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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 August 2002 (01.08.2002)

PCT

(10) International Publication Number
WO 02/059604 A2

(51) International Patent Classification[7]: G01N 33/564, A61K 39/00, A61P 25/00

(21) International Application Number: PCT/GB02/00330

(22) International Filing Date: 25 January 2002 (25.01.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/264,404 26 January 2001 (26.01.2001) US
60/331,647 20 November 2001 (20.11.2001) US

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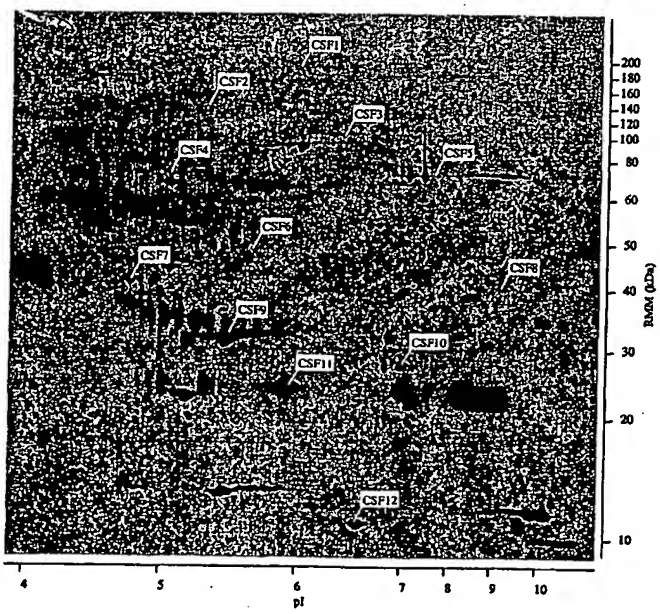
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent

[Continued on next page]

(54) Title: PROTEINS, GENES AND THEIR USE FOR DIAGNOSIS AND TREATMENT OF MULTIPLE SCLEROSIS



(57) Abstract: The present invention provides methods and compositions for screening, diagnosis and prognosis of Multiple Sclerosis, for monitoring the effectiveness of Multiple Sclerosis treatment, identifying patients most likely to respond to a particular therapeutic treatment and for drug development. Multiple Sclerosis-Associated Features (MSFs), detectable by two-dimensional electrophoresis of body fluid e.g. cerebrospinal fluid are described. The invention further provides Multiple Sclerosis-Associated Protein Isoforms (MSPIs) detectable in body fluid e.g. cerebrospinal fluid, preparations comprising isolated MSPIs, antibodies immunospecific for MSPIs, and kits comprising the aforesaid.

WO 02/059604 A2

(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

PROTEINS, GENES AND THEIR USE FOR DIAGNOSIS AND TREATMENT OF MULTIPLE SCLEROSIS

1. INTRODUCTION

The present invention relates to the identification of polypeptides, proteins and protein isoforms that are associated with Multiple Sclerosis and its onset and development, and of genes encoding the same, and to their use for e.g., clinical screening, diagnosis, prognosis, therapy and prophylaxis, as well as for drug screening and drug development.

2. BACKGROUND OF THE INVENTION

Multiple sclerosis (MS) is an inflammatory demyelinating disorder with preservation of the axons and considered the most common cause of neurologic disability in young adults. Although the mean age at onset for MS is 30 years, there are two prevalent age groups. The majority of patients are between 21 and 25 years at onset and a smaller percentage are 41 to 45 years of age. In the western world, more than 80 per 100,000 population are effected (Kurtzke, J.F. (1980) Neurology (N.Y.), 7:261-279). Several twin studies in Canada and the UK revealed that monozygotic twins are concordant in the order of 30%, compared to 2% in dizygotic twins and siblings (Ebers, G.C. et al. (1986) New Engl J Med, 315:1638-42; Mumford, C.J. et al. The British Isles survey of multiple sclerosis in twins. (1994) Neurology, 1004:44, 11-15) and the current evidence suggests that multiple genes may interact to increase susceptibility to MS (Noseworthy (1999) Nature 399:suppl. A40-A47).

While genetics and genotyping may help to define the heritable risk for MS, the utility for diagnosis, prognosis and treatment of MS may be considerably less. It remains still unknown whether MS is a single disease and how it relates to the less common inflammatory-demyelinating central nervous system (CNS) syndromes including neuromyelitis optica, transverse myelitis, Balo's concentric sclerosis, the Marburg variant of acute MS and acute disseminated encephalomyelitis (Noseworthy, Progress in determining the causes and treatment of multiple sclerosis. (1999) Nature 399:suppl. A40-A47). Furthermore, no CNS tissue necessary for any gene expression analysis can be obtained for a living patient under normal circumstances. Proteomic approaches appear most suitable for a molecular dissection of such disease phenotypes in the CNS. The entire CNS is largely inaccessible to meaningful mRNA expression-based analyses of primary human material, since post mortem delays in primary human brain tissue affects mRNAs more readily than proteins (Edgar et al. Molecular Psychiatry 1999, 4, 173-17). Given that cerebrospinal fluid (CSF) bathes the brain, changes in its protein composition may reveal alterations in CNS protein expression pattern causatively or diagnostically linked to the disease. Reasonable amounts of disease specific proteins (DSPs) are secreted or released into body fluids by diseased tissue in the living patient at the onset and/or during progression of the disease. In many cases these alterations in DSPs will be independent of the genetic makeup of the individual and rather directly related to a set of molecular and cellular alterations which contribute to the pathogenic phenotype (Carpenter J Psychiatr Res 1998 32, 191-5).

Postmortem examination of MS patients revealed the presence of multiple lesions

(plaques) in the CNS characterized by demyelination, with relative preservation of axons, as well as gliosis and different degrees of inflammation. Although there are certain sites of predilection including the optic nerves, the spinal cord, and the periventricular regions, any part of the brain or cord can be affected (Lumsden, C.E. (1970) In Vinken P.J. Bruyn, GW, eds., Handbook of Clinical Neurology. Vol. 9. Amsterdam, Noth Holland, P.P. 217-309). In the majority of inflammaory neurological disorders like MS, little is known about a link between changes at a cellular and/or molecular level and nervous system structure and function.

The diagnosis remains a clinical one. Diagnosis requires the demonstration of lesions disseminated in time and space and the exclusion of other conditions that may produce the same clinical picture. Clinical classification of MS known as the Poser criteria include abnormalities of evoked response and Magnetic Resonance Imaging (MRI), and immunologic abnormalities in the CSF (Poser, C.M. et al. (1983) Ann Neurol 13: 227-231). Symptoms of MS at presentation vary among studied populations but include sensory symptoms in 24% of patients, optic neuritis in of 31% of patients, limb weakness in 17% of patients and brain stem and cerebellar symptoms 25% of patients. (Thompson, A.J. et al. (1986) Q.J. Med. 225:69-80). Consequently MS has a wide range of clinical presentations and courses, and the clinical course of any given patient is unpredictable. In the majority of MS patients it begins with a relapsing and remitting course, where episodes of neurological dysfunction last several weeks. Over the course of the disease remissions tend be less than complete and patients pass into a progressive phase (secondary progression). During this phase of the disease patients develop severe irreversible disabilities. About one-third of patients have benign MS, which does not develop secondary progression. Approximately 10% of patients develop progressive disability from onset without relapses and remissions (primary progressive MS). Few biochemical changes have been identified in MS. Consequently the identification and characterization of cellular and/or molecular causative defects and neuropathologies are necessary for improved treatment of neurological disorders. Due to the possibility of worsening or recurrence, speedy diagnosis would be of great benefit, in particular to categorise the patient as follows:

1. Benign versus progressive MS
2. Primary versus secondary progressive MS
3. Specific pathophysiological subtypes of primary and secondary progressive MS

Treatments strategies have three aims: 1, to modify the course of the disease, 2, to affect severity and duration of relapse and 3, symptomatic treatment and neurorehabilitation.

Currently MS has no objective biochemical markers useful for diagnosis and prognosis in living patients. The identification of DSPs in the CSF of MS patients may provide important insights into disease pathology and opportunities for better diagnosis and treatment strategies. Isoelectric focusing of CSF from MS patients revealed the presence of oligoclonal bands in 95% of patients with MS (McLean et al. (1990) Brain, 113:1269-89). However, similar to MRI, this finding is not specific to MS patients and can also be detected in other neurological disorders including Guillain-Barre syndrome, sarcoidosis and chronic menengitis. Therefore, the specificity and the sensitivity of distinguishing individual neurological disorders as well as acute and chronic CNS disease may require the selection of a repertoire of DSPs rather than an individual protein.

Due to the time consuming nature of existing, largely inadequate, tests and their

expense it would be highly desirable to measure a substance or substances in samples of CSF, or other body fluids (e.g. urine, blood, serum) that would lead to a positive diagnosis of MS or that would help to exclude MS from the differential diagnosis.

Therefore, a need exists to identify MS associated proteins as sensitive and specific biomarkers for the diagnosis of MS in living subjects. Additionally, there is a clear need for new therapeutic agents for MS that work quickly, potently, specifically, and with fewer side effects.

3. SUMMARY OF THE INVENTION

The present invention provides methods and compositions for clinical screening, diagnosis and treatment of MS, for monitoring the effectiveness of Multiple Sclerosis treatment, for selecting participants in clinical trials, for identifying patients most likely to respond to a particular therapeutic treatment and for screening and development of drugs for treatment of MS.

A first aspect of the invention provides methods for diagnosis of MS that comprise analyzing a sample of CSF by two-dimensional electrophoresis to detect the presence or level of at least one Multiple Sclerosis-Associated Feature (MSF), e.g., one or more of the MSFs disclosed herein or any combination thereof. These methods are also suitable for clinical screening, prognosis, monitoring the results of therapy, identifying patients most likely to respond to a particular therapeutic treatment, for drug screening and development, and identification of new targets for drug treatment.

A second aspect of the invention provides methods for diagnosis of MS that comprise detecting in a sample of CSF the presence or level of at least one Multiple Sclerosis-Associated Protein Isoform (MSPI), e.g., one or more of the MSPIs disclosed herein or any combination thereof. These methods are also suitable for clinical screening, prognosis, monitoring the results of therapy, identifying patients most likely to respond to a particular therapeutic treatment, drug screening and development, and identification of new targets for drug treatment.

A third aspect of the invention provides antibodies, e.g. monoclonal and polyclonal, chimeric and humanised antibodies capable of immunospecific binding to an MSPI, e.g., an MSPI disclosed herein.

A fourth aspect of the invention provides a preparation comprising an isolated MSPI, i.e., an MSPI free from polypeptides, proteins or protein isoforms having a significantly different isoelectric point or a significantly different apparent molecular weight from the MSPI.

A fifth aspect of the invention provides kits that may be used in the above recited methods and that may comprise single or multiple preparations, or antibodies, together with other reagents, labels, substrates, if needed, and directions for use. The kits may be used for diagnosis of disease, or may be assays for the identification of new diagnostic and/or therapeutic agents.

A sixth aspect of the invention provides methods of treating MS, comprising administering to a subject a therapeutically effective amount of an agent that modulates (e.g., upregulates or downregulates) the expression or activity (e.g. enzymatic or binding activity), or both, of an MSPI in subjects having MS, in order to prevent or delay the onset or development of MS, to prevent or delay the progression of MS, or to ameliorate the symptoms of MS.

A seventh aspect of the invention provides methods of screening for agents that modulate (e.g., upregulate or downregulate) a characteristic of, e.g., the expression or the enzymatic or binding activity, of a MSF, MSPI, a MSPI analog, or a MSPI-related polypeptide.

4. BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is an image obtained from 2-dimensional electrophoresis of CSF, which has been annotated to identify twelve landmark features, designated CSF1 to CSF12.

Figure 2 is a flow chart depicting the characterization of an MSF and relationship of a MSF and MSPI. A MSF may be further characterized as or by an MSPI having a particular peptide sequence associated with its pI and MW. As depicted herein, a MSFmay comprise one or more MSPIs, which have indistinguishable pIs and MWs using the Preferred Technology, but which have distinct peptide sequences. The peptide sequence(s) of the MSPI can be utilized to search database(s) for previously identified proteins comprising such peptide sequence(s). It can be ascertained whether a commercially available antibody exists that may recognize the previously identified protein and/or a member of its protein family.

5. DETAILED DESCRIPTION OF THE INVENTION

The invention described in detail below provides methods and compositions for clinical screening and diagnosis of Multiple Sclerosis in a mammalian subject for identifying patients most likely to respond to a particular therapeutic treatment, for monitoring the results of Multiple Sclerosis therapy, for drug screening and drug development. The invention also encompasses the administration of therapeutic compositions to a mammalian subject to treat or prevent Multiple Sclerosis. The mammalian subject may be a non-human mammal, but is preferably human, more preferably a human adult, i.e. a human subject at least 21 years old. For clarity of disclosure, and not by way of limitation, the invention will be described with respect to the analysis of CSF samples. However, as one skilled in the art will appreciate, the assays and techniques described below can be applied to other types of samples, including a body fluid (for example but without limitation: blood, serum, plasma, saliva or urine), a tissue sample from a subject at risk of having or developing MS (e.g. a biopsy such as a brain biopsy) or homogenate thereof. The methods and compositions of the present invention are useful for screening and diagnosis of a living subject, but may also be used for postmortem diagnosis in a subject, for example, to identify family members of the subject who are at risk of developing the same disease.

The following definitions are provided to assist in the review of the instant disclosure.

5.1 DEFINITIONS

"Feature" refers to a spot identified in a 2D gel, and the term "Multiple Sclerosis - Associated Feature" (MSF) refers to a feature that is differentially present in a first sample or sample set from a subject having MS compared with a second sample or sample set from a subject free from MS. A feature or spot identified in a 2D gel is characterized by its isoelectric point (pI) and apparent molecular weight (MW) as determined by 2D gel electrophoresis, particularly utilizing the Preferred Technology described herein. As used herein, a feature is "differentially present" in a first sample or sample set with respect to a second sample or sample set when a method for detecting the said feature (*e.g.*, 2D electrophoresis) gives a

different signal when applied to the first and second samples or sample sets. A MSF, (or a Protein Isoform, i.e. MSPI, as defined *infra*) is "increased" in the first sample or sample set with respect to the second sample or sample set if the method of detection indicates that the MSF, or MSPI is more abundant in the first sample or sample set than in the second sample or sample set, or if the MSF, or MSPI is detectable in the first sample or sample set and substantially undetectable in the second sample or sample set. Conversely, a MSF, or MSPI is "decreased" in the first sample or sample set with respect to the second sample or sample set if the method of detection indicates that the MSF, or MSPI is less abundant in the first sample or sample set than in the second sample or sample set or if the MSF, or MSPI is undetectable in the first sample or sample set and detectable in the second sample or sample set.

Particularly, the relative abundance of a feature in the two samples or sample sets is determined in reference to its normalized signal, in two steps. First, the signal obtained upon detecting the feature in a first sample or sample set is normalized by reference to a suitable background parameter, *e.g.*, (a) to the total protein in the sample being analyzed (*e.g.*, total protein loaded onto a gel); (b) to an Expression Reference Feature (ERF) i.e., a feature whose abundance is substantially invariant, within the limits of variability of the Preferred Technology, in the population of subjects being examined, *e.g.* the ERFs disclosed in Table III, or (c) more preferably to the total signal detected as the sum of each of all proteins in the sample.

Secondly, the normalized signal for the feature in the first sample or sample set is compared with the normalized signal for the same feature in the second sample or sample set in order to identify features that are "differentially present" in the first sample or sample set with respect to the second sample or sample set.

"Fold change" includes "fold increase" and "fold decrease" and refers to the relative increase or decrease in abundance of a MSF or the relative increase or decrease in expression or activity of a polypeptide (*e.g.* a MSPI, as defined *infra.*) in a first sample or sample set compared to a second sample or sample set. A MSF or polypeptide fold change may be measured by any technique known to those of skill in the art, albeit the observed increase or decrease will vary depending upon the technique used. Preferably, fold change is determined herein as described in the Examples *infra.*

"Multiple Sclerosis-Associated Protein Isoform" (MSPI) refers to a polypeptide that is differentially present in a first sample or sample set from a subject having MS compared with a second sample or sample set from a subject free from MS. As used herein, a MSPI is "differentially present" in a first sample or sample set with respect to a second sample or sample set when a method for detecting the said feature, (*e.g.*, 2D electrophoresis or immunoassay) gives a different signal when applied to the first and second samples or sample sets (as described above in relation to MSFs). A MSPI is characterised by one or more peptide sequences of which it is comprised, and further by a pI and MW, preferably determined by 2D electrophoresis, particularly utilising the Preferred Technology as described herein. Typically, MSPIs are identified or characterised by the amino acid sequencing of MSFs (Figure 2).

An MSPI is characterized as, or by, a particular peptide sequence associated with its pI and MW. As depicted herein, a MSF may comprise one or more MSPI(s), which have indistinguishable pIs and MWs using the Preferred Technology, but which have distinct peptide sequences. The peptide sequence(s) of the MSPI can be utilized to search database(s) for

previously identified proteins comprising such peptide sequence(s). In some instances, it can be ascertained whether a commercially available antibody exists which may recognize the previously identified protein and/or a variant thereof. Prefereably the MSPI corresponds to the previously identified protein, or be a variant of the previously identified protein.

"Variant" as used herein refers to a polypeptide which is a member of a family of polypeptides that are encoded by a single gene or from a gene sequence within a family of related genes and which differ in their pI or MW, or both. Such variants can differ in their amino acid composition (*e.g.* as a result of alternative mRNA or premRNA processing, *e.g.* alternative splicing or limited proteolysis) and in addition, or in the alternative, may arise from differential post-translational modification (*e.g.*, glycosylation, acylation, phosphorylation).

"Modulate" in reference to expression or activity of a MSF, MSPI or MSPI-related polypeptide refers to any change, *e.g.*, upregulation or downregulation, increase or decrease, of the expression or activity of the MSF, MSPI or MSPI-related polypeptide. Those skilled in the art, based on the present disclosure, will understand that such modulation can be determined by assays known to those of skill in the art.

"MSPI analog" refers to a polypeptide that possesses similar or identical function(s) as a MSPI but need not necessarily comprise an amino acid sequence that is similar or identical to the amino acid sequence of the MSPI, or possess a structure that is similar or identical to that of the MSPI. As used herein, an amino acid sequence of a polypeptide is "similar" to that of a MSPI if it satisfies at least one of the following criteria: (a) the polypeptide has an amino acid sequence that is at least 30% (more preferably, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99%) identical to the amino acid sequence of the MSPI; (b) the polypeptide is encoded by a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence encoding at least 5 amino acid residues (more preferably, at least 10 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, or at least 150 amino acid residues) of the MSPI; or (c) the polypeptide is encoded by a nucleotide sequence that is at least 30% (more preferably, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99%) identical to the nucleotide sequence encoding the MSPI. As used herein, a polypeptide with "similar structure" to that of a MSPI refers to a polypeptide that has a similar secondary, tertiary or quarternary structure as that of the MSPI. The structure of a polypeptide can determined by methods known to those skilled in the art, including but not limited to, X-ray crystallography, nuclear magnetic resonance, and crystallographic electron microscopy.

"MSPI fusion protein" refers to a polypeptide that comprises (i) an amino acid sequence of an MSPI, MSPI fragment, MSPI-related polypeptide or a fragment of an MSPI-related polypeptide and (ii) an amino acid sequence of a heterologous polypeptide (i.e., a non-MSPI, non-MSPI fragment or non-MSPI-related polypeptide).

"MSPI homolog" refers to a polypeptide that comprises an amino acid sequence similar to that of a MSPI but does not necessarily possess a similar or identical function as the

MSPI.

"MSPI ortholog" refers to a non-human polypeptide that (i) comprises an amino acid sequence similar to that of a MSPI and (ii) possesses a similar or identical function to that of the MSPI.

"MSPI-related polypeptide" refers to a MSPI homolog, a MSPI analog, a variant of MSPI, a MSPI ortholog, or any combination thereof.

"Chimeric Antibody" refers to a molecule in which different portions are derived from different animal species, such as those having a human immunoglobulin constant region and a variable region derived from a murine mAb. (See, *e.g.*, Cabilly et al., U.S. Patent No. 4,816,567; and Boss et al., U.S. Patent No. 4,816397, which are incorporated herein by reference in their entirety.)). For example, a portion of the antibody may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric antibodies.

"Humanised Antibody" refers to a molecule from non-human species having one or more complementarily determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule.

"Derivative" refers to a polypeptide that comprises an amino acid sequence of a second polypeptide that has been altered by the introduction of at least one amino acid residue substitution, deletion or addition. The derivative polypeptide possesses a similar or identical function as the second polypeptide.

"Fragment" refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 amino acid residues (preferably, at least 10 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, at least 150 amino acid residues, at least 175 amino acid residues, at least 200 amino acid residues, or at least 250 amino acid residues) of the amino acid sequence of a second polypeptide. Prefereably the fragment of a MSPI possesses the functional activity of the MSPI.

The "percent identity" of two amino acid sequences or of two nucleic acid sequences can be or is generally determined by aligning the sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in either sequences for best alignment with the other sequence) and comparing the amino acid residues or nucleotides at corresponding positions. The "best alignment" is an alignment of two sequences that results in the highest percent identity. The percent identity is determined by the number of identical amino acid residues or nucleotides in the sequences being compared (i.e., % identity = # of identical positions/total # of positions x 100).

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm known to those of skill in the art. An example of a mathematical algorithm for comparing two sequences is the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-2268, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5877. The NBLAST and XBLAST programs of Altschul, et al. (1990) J. Mol. Biol. 215:403-410 have incorporated such an algorithm. BLAST nucleotide searches can be performed with the NBLAST program, score = 100,

wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecule of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecule of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997) Nucleic Acids Res. 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used.

Another example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). The ALIGN program (version 2.0) which is part of the GCG sequence alignment software package has incorporated such an algorithm. Other algorithms for sequence analysis known in the art include ADVANCE and ADAM as described in Torellis and Robotti (1994) Comput. Appl. Biosci., 10 :3-5; and FASTA described in Pearson and Lipman (1988) Proc. Natl. Acad. Sci. 85:2444-8. Within FASTA, ktup is a control option that sets the sensitivity and speed of the search.

"Diagnosis" refers to diagnosis, prognosis, monitoring, characterizing, selecting patients, including participants in clinical trials, and identifying patients at risk for or having a particular disorder or those most likely to respond to a particular therapeutic treatment, or for assessing or monitoring a patient's response to a particular therapeutic treatment.

"Treatment" refers to therapy, prevention and prophylaxis and particularly refers to the administration of medicine or the performance of medical procedures with respect to a patient, for either prophylaxis (prevention) or to cure the infirmity or malady in the instance where the patient is afflicted.

"Agent" refers to all materials that may be used to prepare pharmaceutical and diagnostic compositions, or that may be compounds, agonists, antagonists, nucleic acids, polypeptides, fragments, isoforms, variants, or other materials that may be used independently for such purposes, all in accordance with the present invention.

"Highly stringent conditions" refers to hybridization to filter-bound DNA in 0.5 M NaHPO4, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C (Ausubel F.M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3; incorporated herein by reference in its entirety.)

For some applications, less stringent conditions for duplex formation are required. As used herein "moderately stringent conditions" refers to washing in 0.2xSSC/0.1% SDS at 42°C (Ausubel et al., 1989, supra).

"Cerebrospinal fluid (CSF)" refers to the fluid that surrounds the bulk of the CNS, as described in Physiological Basis of Medical Practice (J.B. West, ed., Williams and Wilkins, Baltimore, MD 1985). CSF includes ventricular CSF and lumbar CSF.

"Serum" refers to the supernatant fluid produced by clotting and centrifugal sedimentation of a blood sample.

"Plasma" refers to the supernatant fluid produced by inhibition of clotting (for example, by citrate or EDTA) and centrifugal sedimentation of a blood sample.

"Blood" as used herein includes serum and plasma.

"Two-dimensional electrophoresis" (2D-electrophoresis) means a technique

comprising isoelectric focusing, followed by denaturing electrophoresis; this generates a two-dimensional gel (2D-gel) containing a plurality of separated proteins.

5.2 THE "PREFERRED TECHNOLOGY"

Preferably, the step of denaturing electrophoresis uses polyacrylamide electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). Especially preferred are the highly accurate and automatable methods and apparatus ("the Preferred Technology") described in WO 98/23950 and in U.S. Patent Nos 6,064,654, and 6,278.794, each of which is incorporated herein by reference in its entirety with particular reference to the protocol at pages 23-35 of WO 98/23950. Briefly, the Preferred Technology provides efficient, computer-assisted methods and apparatus for identifying, selecting and characterising biomolecules (e.g. proteins, including glycoproteins) in a biological sample. A two-dimensional array is generated by separating biomolecules on a two-dimensional gel according to their electrophoretic mobility and isoelectric point. A computer-generated digital profile of the array is generated, representing the identity, apparent molecular weight, isoelectric point, and relative abundance of a plurality of biomolecules detected in the two-dimensional array, thereby permitting computer-mediated comparison of profiles from multiple biological samples, as well as computer aided excision of separated proteins of interest.

A preferred scanner for detecting fluorescently labeled proteins is described in WO 96/36882 and in the Ph.D. thesis of David A. Basiji, entitled "Development of a High-throughput Fluorescence Scanner Employing Internal Reflection Optics and Phase-sensitive Detection (Total Internal Reflection, Electrophoresis)", University of Washington (1997), Volume 58/12-B of Dissertation Abstracts International, page 6686, the contents of each of which are incorporated herein by reference. These documents describe an image scanner designed specifically for automated, integrated operation at high speeds. The scanner can image gels that have been stained with fluorescent dyes or silver stains, as well as storage phosphor screens. The Basiji thesis provides a phase-sensitive detection system for discriminating modulated fluorescence from baseline noise due to laser scatter or homogeneous fluorescence, but the scanner can also be operated in a non-phase-sensitive mode. This phase-sensitive detection capability would increase the sensitivity of the instrument by an order of magnitude or more compared to conventional fluorescence imaging systems. The increased sensitivity would reduce the sample-preparation load on the upstream instruments while the enhanced image quality simplifies image analysis downstream in the process.

A more highly preferred scanner is a modified version of the above described scanner. In the preferred scanner, the gel is transported through the scanner on a precision lead-screw drive system. This is preferable to laying the glass plate on the belt-driven system that is described in the Basiji thesis, as it provides a reproducible means of accurately transporting the gel past the imaging optics.

In the preferred scanner, the gel is secured against three alignment stops that rigidly hold the glass plate in a known position. By doing this in conjunction with the above precision transport system, the absolute position of the gel can be predicted and recorded. This ensures that co-ordinates of each feature on the gel can be determined more accurately and communicated, if desired, to a cutting robot for excision of the feature. In the preferred

scanner, the carrier that holds the gel has four integral fluorescent markers for use to correct the image geometry. These markers are a quality control feature that confirms that the scanning has been performed correctly.

In comparison to the scanner described in the Basiji thesis, the optical components of the preferred scanner have been inverted. In the preferred scanner, the laser, mirror, waveguide and other optical components are above the glass plate being scanned. The scanner described in the Basiji thesis has these components underneath. In the preferred scanner, the glass plate is mounted onto the scanner gel side down, so that the optical path remains through the glass plate. By doing this, any particles of gel that may break away from the glass plate will fall onto the base of the instrument rather than into the optics. This does not affect the functionality of the system, but increases its reliability.

Still more preferred is a modified version of the preferred scanner, in which the signal output is digitised to the full 16-bit data without any peak saturation or without square root encoding of the signal. A compensation algorithm has also been applied to correct for any variation in detection sensitivity along the path of the scanning beam. This variation is due to anomalies in the optics and differences in collection efficiency across the waveguide. A calibration is performed using a perspex plate with an even fluorescence throughout. The data received from a scan of this plate are used to determine the multiplication factors needed to increase the signal from each pixel level to a target level. These factors are then used in subsequent scans of gels to remove any internal optical variations.

5.3 MULTIPLE SCLEROSIS-ASSOCIATED FEATURES (MSFs)

In one aspect of the invention, two-dimensional electrophoresis is used to analyze CSF from a subject, preferably a living subject, in order to detect or quantify the expression of one or more Multiple Sclerosis-Associated Features (MSFs) for screening, prevention or diagnosis of MS, to determine the prognosis of a subject having MS, to monitor progression of MS, to monitor the effectiveness of MS therapy, for identifying patients most likely to respond to a particular therapeutic treatment, or for drug development.

By way of example and not of limitation, using the Preferred Technology, a number of samples from subjects having MS and samples from subjects free from MS are separated by two-dimensional electrophoresis, and the fluorescent digital images of the resulting gels are matched to a chosen representative primary master gel image. This process allows any gel feature, characterised by its pI and MW, to be identified and examined on any gel of the study. In particular, the amount of protein present in a given feature can be measured in each gel; this feature abundance can be averaged amongst gels from similar samples (e.g. gels from samples from subjects having MS). Finally, statistical analyses can be conducted on the thus created sample sets, in order to compare 2 or more sample sets to each other.

The MSFs disclosed herein have been identified by comparing CSF samples from subjects having MS against CSF samples from subjects free from MS. Subjects free from MS include subjects with no known disease or condition (normal subjects) and subjects with diseases other than MS.

Two groups of MSFs have been identified through the methods and apparatus of the Preferred Technology. The first group consists of MSFs that are decreased in the CSF of subjects having MS as compared with the CSF of subjects free from MS, where the differential

presence is significant. These MSFs can be described by apparent molecular weight (MW) and isoelectric point (pI) as provided in Table I.

Table I. MSFs Decreased in CSF of Subjects Having MS

MSF#	pI	MW (Da)	Fold Decrease
MSF-1	8.75	21021	>100
MSF-2	5.62	34394	>100
MSF-3	9.75	11627	>100
MSF-4	6.24	102603	>100
MSF-5	6.18	105482	>100
MSF-6	5.91	21888	>100
MSF-7	5.52	56560	>100
MSF-8	5.49	34394	>100
MSF-9	6.01	184426	>100
MSF-10	6.06	184426	86.63
MSF-11	5.96	185225	83.95
MSF-12	5.41	51566	79.92
MSF-13	5.92	185225	74.98
MSF-14	6.80	32080	65.52
MSF-15	5.88	186027	53.51
MSF-16	6.14	186027	49.41
MSF-17	5.51	12598	46.66
MSF-18	5.79	186832	43.72
MSF-20	5.50	20607	39.71
MSF-21	4.70	43626	39.47
MSF-22	5.75	186832	35.45
MSF-23	5.67	178932	35.06
MSF-24	6.18	187641	34.05
MSF-25	6.23	48975	32.70
MSF-26	6.18	178932	29.18
MSF-27	6.11	126160	26.38
MSF-28	6.23	186832	25.16
MSF-29	6.10	184426	23.38
MSF-30	7.82	31104	22.71
MSF-31	5.84	186832	22.09
MSF-32	6.03	135312	21.97
MSF-33	4.89	52993	21.83
MSF-34	5.59	21103	21.54
MSF-35	6.24	74941	20.84
MSF-36	5.93	49874	20.74
MSF-37	5.30	179707	20.61
MSF-38	6.28	87995	20.13
MSF-39	4.82	98505	19.20
MSF-40	5.35	11955	17.97
MSF-41	5.79	22491	17.70
MSF-42	5.98	64540	17.13
MSF-43	5.54	37852	16.38
MSF-44	6.28	27735	16.05
MSF-45	4.70	64736	15.87
MSF-46	6.13	178161	14.86

MSF#	pI	MW (Da)	Fold Decrease
MSF-47	5.49	64150	13.99
MSF-48	4.77	99610	12.90
MSF-49	4.54	74941	12.85
MSF-50	5.83	111485	12.58
MSF-51	7.48	11668	12.15
MSF-52	5.72	146050	11.63
MSF-53	4.99	40463	11.59
MSF-54	8.64	20604	11.51
MSF-55	5.65	150534	10.77
MSF-56	5.49	158545	10.52
MSF-57	9.92	29162	10.44
MSF-58	5.66	186832	10.26
MSF-59	6.23	178932	10.26
MSF-60	5.60	150534	10.25
MSF-61	6.35	164223	9.93
MSF-62	6.52	175109	9.70
MSF-63	5.00	171366	9.60
MSF-64	6.32	178932	9.40
MSF-65	6.18	136566	9.21
MSF-66	5.49	98505	9.01
MSF-67	5.16	53159	8.86
MSF-69	4.51	126852	8.79
MSF-70	6.15	89080	8.73
MSF-71	5.56	85203	8.73
MSF-72	5.09	156672	8.58
MSF-73	7.26	33226	8.46
MSF-75	10.06	11587	8.39
MSF-76	5.72	151186	8.30
MSF-77	6.38	177393	8.14
MSF-78	6.25	33313	8.09
MSF-79	5.48	85680	8.08
MSF-80	6.06	60926	7.60
MSF-81	5.94	42899	7.16
MSF-82	5.72	100168	6.73
MSF-83	5.51	44365	6.71
MSF-84	5.53	99610	6.70
MSF-85	5.39	38741	6.34
MSF-86	4.54	27735	6.23
MSF-87	5.29	125687	5.86
MSF-88	5.57	80579	5.62
MSF-89	5.58	63762	5.61
MSF-90	6.08	93159	5.38
MSF-91	6.26	21818	5.34
MSF-92	5.54	150534	5.13
MSF-93	5.70	34998	5.12
MSF-94	5.63	178161	5.08
MSF-95	5.28	52673	5.01
MSF-96	5.80	91613	4.99
MSF-97	5.71	91103	4.86
MSF-98	5.54	48679	4.80
MSF-99	5.43	112518	4.62

MSF#	pI	MW (Da)	Fold Decrease
MSF-100	5.99	67749	4.54
MSF-101	5.59	178161	4.54
MSF-102	5.95	34494	4.49
MSF-104	6.29	100168	4.40
MSF-105	4.84	25658	4.37
MSF-106	8.13	68368	4.33
MSF-107	4.95	52196	4.33
MSF-108	6.05	47450	4.25
MSF-109	5.32	35100	4.15
MSF-110	6.17	93159	4.14
MSF-111	5.67	48092	4.06
MSF-112	5.48	55124	4.06
MSF-113	6.12	101661	3.96
MSF-114	5.28	154488	3.91
MSF-115	5.85	29583	3.88
MSF-116	5.39	142313	3.85
MSF-117	5.00	132839	3.85
MSF-119	6.00	49723	3.84
MSF-120	5.78	72071	3.83
MSF-121	4.97	18472	3.79
MSF-122	5.03	17230	3.76
MSF-123	5.89	91613	3.76
MSF-125	4.46	29058	3.66
MSF-126	5.86	17929	3.63
MSF-127	5.62	54294	3.63
MSF-128	5.57	142313	3.61
MSF-130	4.88	95795	3.56
MSF-131	5.03	50944	3.55
MSF-132	5.16	41028	3.51
MSF-133	5.87	67575	3.51
MSF-134	5.98	90092	3.50
MSF-135	5.18	180485	3.45
MSF-136	4.70	37742	3.44
MSF-137	5.97	56994	3.43
MSF-139	6.06	28850	3.42
MSF-140	6.95	19478	3.39
MSF-141	5.77	52354	3.35
MSF-142	5.77	54791	3.35
MSF-143	5.95	19673	3.30
MSF-144	5.48	46191	3.29
MSF-145	5.40	151841	3.29
MSF-147	7.10	73878	3.26
MSF-148	5.07	131619	3.23
MSF-149	6.21	101661	3.22
MSF-150	5.50	151186	3.20
MSF-151	9.39	11427	3.18
MSF-152	5.55	60192	3.16
MSF-153	5.79	20456	3.15
MSF-154	5.28	142929	3.14
MSF-155	6.33	49273	3.13
MSF-156	5.85	48238	3.10

MSF#	pI	MW (Da)	Fold Decrease
MSF-157	5.83	27935	3.09
MSF-158	5.88	64150	3.06
MSF-159	6.37	101661	3.03
MSF-160	5.54	47450	3.02
MSF-161	6.25	55124	3.01
MSF-163	5.32	142929	2.99
MSF-165	5.77	50178	2.95
MSF-167	6.17	48679	2.94
MSF-168	5.36	98505	2.93
MSF-169	5.12	139109	2.82
MSF-170	5.31	156503	2.82
MSF-171	4.99	15072	2.73
MSF-172	5.56	54294	2.72
MSF-173	5.85	62512	2.65
MSF-174	5.95	36556	2.63
MSF-175	4.98	100168	2.63
MSF-176	5.43	48238	2.63
MSF-177	7.67	68368	2.63
MSF-178	8.47	22644	2.60
MSF-179	5.60	46036	2.54
MSF-180	6.21	36134	2.47
MSF-181	5.13	21638	2.46
MSF-182	5.62	52038	2.46
MSF-183	8.76	18350	2.44
MSF-184	6.30	50790	2.43
MSF-185	4.81	18657	2.42
MSF-186	6.81	48827	2.38
MSF-187	6.01	29689	2.37
MSF-188	5.59	100168	2.37
MSF-189	5.74	65725	2.34
MSF-190	5.92	16835	2.33
MSF-191	6.24	87097	2.33
MSF-192	5.49	19869	2.33
MSF-193	5.90	23795	2.32
MSF-194	6.91	116746	2.29
MSF-195	5.45	66932	2.28
MSF-196	8.22	51254	2.26
MSF-197	5.11	50790	2.23
MSF-198	5.59	20201	2.17
MSF-199	5.43	49573	2.16
MSF-200	7.14	116746	2.15
MSF-201	6.65	12463	2.14
MSF-202	4.93	81483	2.14
MSF-203	6.00	38405	2.13
MSF-204	5.82	67210	2.10
MSF-205	5.28	72474	2.09
MSF-206	6.47	28747	2.07
MSF-207	4.94	134070	2.06
MSF-208	5.37	123390	2.02
MSF-209	5.49	24374	1.96
MSF-210	5.66	50026	1.95

MSF#	pI	MW (Da)	Fold Decrease
MSF-211	5.19	50178	1.94
MSF-212	5.79	64150	1.89
MSF-213	5.83	13375	1.87
MSF-215	5.11	35613	1.86
MSF-216	9.43	68368	1.82
MSF-217	9.04	11790	1.81
MSF-218	5.08	91613	1.81
MSF-219	7.01	117828	1.74
MSF-220	4.56	46503	1.74
MSF-221	5.55	66125	1.70
MSF-222	5.54	49573	1.69
MSF-223	5.62	50026	1.65
MSF-224	5.07	65130	1.64
MSF-225	4.64	122257	1.61
MSF-226	4.81	50178	1.60
MSF-227	7.46	118510	1.59
MSF-228	4.95	44515	1.57
MSF-229	6.07	79685	1.54
MSF-230	6.98	97467	1.53
MSF-231	5.30	49423	1.53
MSF-232	7.27	27636	1.52
MSF-233	7.35	36450	1.46
MSF-234	5.37	24470	1.43
MSF-235	5.36	20134	1.42
MSF-236	5.20	72071	1.38

The second group consists of MSFs that are increased in the CSF of subjects having MS as compared with the CSF of subjects free from MS where the differential presence is significant. These MSFs can be described by MW and pI as provided in Table II:

Table II. MSFs Increased in CSF of Subjects Having MS

MSF#	pI	MW (Da)	Fold Increase
MSF-237	10.15	11421	>100
MSF-238	8.26	11328	>100
MSF-239	6.65	14399	68.46
MSF-239	6.65	14399	68.46
MSF-240	7.73	27538	64.71
MSF-241	6.10	86456	41.27
MSF-242	7.30	25465	25.21
MSF-243	6.01	12948	22.91
MSF-244	5.27	11037	16.46
MSF-245	6.21	80579	16.40
MSF-246	8.78	15019	15.96
MSF-247	6.28	27245	15.02
MSF-248	7.19	30921	14.98
MSF-250	4.33	42755	12.76
MSF-251	7.60	81385	12.75
MSF-252	5.62	26380	11.74

MSF#	pI	MW (Da)	Fold Increase
MSF-253	6.30	17837	11.68
MSF-254	4.28	11270	11.29
MSF-257	4.64	13792	10.95
MSF-259	5.25	27594	10.73
MSF-260	6.06	78111	10.45
MSF-261	4.86	44065	10.19
MSF-262	4.40	60275	10.15
MSF-263	7.50	45269	10.12
MSF-264	7.93	66125	10.06
MSF-265	6.56	20744	9.93
MSF-266	6.54	13783	9.64
MSF-267	5.54	21908	9.63
MSF-268	8.85	13625	9.38
MSF-269	5.49	28193	9.27
MSF-270	5.74	32454	9.23
MSF-271	6.43	45269	8.92
MSF-273	4.94	16019	8.81
MSF-274	9.80	23795	8.15
MSF-276	7.32	13812	7.85
MSF-277	6.29	80131	7.59
MSF-278	4.90	82867	7.48
MSF-279	6.69	24664	7.36
MSF-280	6.06	33713	7.36
MSF-281	6.37	80131	7.23
MSF-282	6.28	27116	7.14
MSF-283	4.78	43626	7.03
MSF-284	6.43	26066	6.88
MSF-285	5.75	27835	6.83
MSF-287	6.65	17872	6.72
MSF-289	6.74	54791	6.39
MSF-290	4.36	20951	6.36
MSF-291	4.33	105354	6.33
MSF-292	5.76	13128	6.31
MSF-294	8.76	15213	6.29
MSF-295	4.32	36134	6.23
MSF-296	4.94	20403	6.22
MSF-298	7.51	24762	6.09
MSF-299	8.12	54806	6.03
MSF-300	4.37	40820	5.84
MSF-301	5.58	32266	5.75
MSF-302	4.28	21301	3.96
MSF-303	7.84	65034	3.75
MSF-305	9.04	21021	3.49
MSF-306	6.86	50636	3.47
MSF-307	7.48	59646	3.38
MSF-308	7.78	55797	3.32
MSF-310	5.01	13359	2.69
MSF-311	5.41	58040	2.67
MSF-312	7.39	40510	2.66
MSF-313	5.58	24087	2.49
MSF-314	4.84	26797	2.33

MSF#	pI	MW (Da)	Fold Increase
MSF-316	5.51	31894	2.13
MSF-318	4.57	13499	2.00
MSF-319	6.72	59646	1.94
MSF-320	8.16	24182	1.91
MSF-321	6.37	21021	1.84
MSF-322	5.21	22551	1.75
MSF-323	5.21	31216	1.73
MSF-324	7.10	23117	1.60
MSF-325	4.05	43334	1.44

For any given MSF, the signal obtained upon analyzing CSF from subjects having MS relative to the signal obtained upon analyzing CSF from subjects free from MS will depend upon the particular analytical protocol and detection technique that is used. Accordingly, the present invention contemplates that each laboratory will, based on the present description, establish a reference range for each MSF in subjects free from MS according to the analytical protocol and detection technique in use, as is conventional in the diagnostic art. Preferably, at least one control positive CSF sample from a subject known to have MS or at least one control negative CSF sample from a subject known to be free from MS (and more preferably both positive and negative control samples) are included in each batch of test samples analyzed. In one embodiment, the level of expression of a feature is determined relative to a background value, which is defined as the level of signal obtained from a proximal region of the image that (a) is equivalent in area to the particular feature in question; and (b) contains no discernable protein feature.

In a preferred embodiment, the signal associated with an MSF in the CSF of a subject (e.g., a subject suspected of having or known to have MS is normalised with reference to one or more ERFs detected in the same 2D gel. As will be apparent to one of ordinary skill in the art, such ERFs may readily be determined by comparing different samples using the Preferred Technology. Suitable ERFs include (but are not limited to) thos described in Table III.

Table III. Expression Reference Features

ERF#	pI	MW (Da)
ERF-1	5.28	57515
ERF-2	5.25	20268
ERF-3	8.76	16126

As those of skill in the art will readily appreciate, the apparent MW and pI of a given feature or protein isoform will vary to some extent depending on the precise protocol used for each step of the 2D electrophoresis and for landmark matching (as described in section 6.1.8 *infra*). As used herein, the terms "MW" and "pI" are defined, respectively, to mean the apparent molecular weight and the apparent isoelectric point of a feature or protein isoform as measured in exact accordance with the Reference Protocol identified in Section 6 below. When the Reference Protocol is followed and when samples are run in duplicate or a higher

number of replicates, variation in the measured mean pI of an MSF or MSPI is typically less than 3% and variation in the measured mean MW of an MSF or MSPI is typically less than 5%. Where the skilled artisan wishes to deviate from the Reference Protocol, calibration experiments should be performed to compare the MW and pI for each MSF or protein isoform as detected (a) by the Reference Protocol and (b) by the deviant protocol.

MSFs can be used for detection, diagnosis, or monitoring of MS, or for identifying patients most likely to respond to a specific therapeutic treatment, or for drug development. In one embodiment of the invention, a first sample of body fluid from a subject (e.g., a subject suspected of having MS) is analyzed by 2D electrophoresis for quantitative detection of one or more of the MSFs as defined in Table I. A decreased abundance of said one or more of these MSFs in the first sample from the subject relative to a second sample from a subject or subjects free from MS (e.g., a control sample or a previously determined reference range) indicates the presence of MS.

In another embodiment of the invention, a first sample of body fluid from a subject is analyzed by 2D electrophoresis for the quantitative detection of one or more of the MSFs as defined in Table II. An increased abundance of said one or more MSFs in the first sample from the subject relative to a second sample from a subject or subjects free from MS (e.g., a control sample or a previously determined reference range) indicates the presence of MS.

In yet another embodiment, a first sample of body fliud from a subject is analyzed by 2D electrophoresis for quantitative detection of (a) one or more MSFs or any combination of them, whose decreased abundance indicates the presence of MS, i.e., the MSFs as defined in Table I; and (b) one or more MSFs or any combination of them, whose increased abundance indicates the presence of MS i.e., MSFs as defined in Table II.

In yet another embodiment of the invention, a first sample of body fluid from a subject is analyzed by 2D electrophoresis for quantitative detection of one or more of the MSFs as defined in Table I or Table II:, wherein the ratio of the one or more MSFs relative to an Expression Reference Feature (ERF) indicates whether MS is present. In a specific embodiment, a decrease in one or more MSF/ERF ratios in a first sample relative to the MSF/ERF ratios in a second sample or a reference range indicates the presence of MS; i.e. the MSFs as defined in Table I are suitable MSFs for this purpose. In another specific embodiment, an increase in one or more MSF/ERF ratios in a first sample relative to the MSF/ERF ratios in a second sample or a reference range indicates the presence of MS; the MSFs as defined in Table II are suitable MSFs for this purpose.

In a further embodiment of the invention, a first sample of body fluid from a subject is analyzed by 2D electrophoresis for quantitative detection of (a) one or more MSFs, or any combination of them, whose decreased MSF/ERF ratio(s) in a first sample relative to the MSF/ERF ratio(s) in a second sample indicates the presence of MS, i.e., the MSFs as defined in Table I; (b) one or more MSFs, or any combination of them, whose increased MSF/ERF ratio(s) in a first sample relative to the MSF/ERF ratio(s) in a second sample indicates the presence of MS, i.e., the MSFs as defined in Table II.

In a preferred embodiment, CSF from a subject is analyzed for quantitative detection of a plurality of MSFs.

5.4 MULTIPLE SCLEROSIS-ASSOCIATED PROTEIN ISOFORMS (MSPIs)

In another aspect of the invention, a sample of body fluid from a subject, preferably a living subject, is analyzed for quantitative detection of one or more Multiple Sclerosis-Associated Protein Isoforms (MSPIs) for screening or diagnosis of MS, to monitor the effectiveness of MS therapy, for identifying patients most likely to respond to a particular therapeutic treatment or for drug development. As is well known in the art, a given protein may be expressed as variants that differ in their amino acid composition (e.g. as a result of alternative mRNA or premRNA processing, e.g. alternative splicing or limited proteolysis) or as a result of differential post-translational modification (e.g., glycosylation, phosphorylation, acylation), or both, so that proteins of identical amino acid sequence can differ in their pI, MW, or both. It follows that differential presence of a protein isoform does not require differential expression of the gene encoding the protein in question. As used herein, the term "Multiple Sclerosis-Associated Protein Isoform" refers to a polypeptide that is differentially present in a first sample of body fluid from a subject having MS compared with second sample from a subject free from MS.

Two groups of MSPIs have been identified by amino acid sequencing of MSFs. MSPIs were isolated, subjected to proteolysis, and analyzed by mass spectrometry using the methods and apparatus of the Preferred Technology. One skilled in the art can identify sequence information from proteins analyzed by mass spectrometry and/or tandem mass spectrometry using various spectral interpretation methods and database searching tools. Examples of some of these methods and tools can be found at the Swiss Institute of Bioinformatics web site at http://www.expasy.com/, and the European Molecular Biology Laboratory web site at http://www.narrador.embl-heidelberg.de/GroupPages/PageLink/peptidesearchpage.html. Identification of MSPIs was performed primarily using the SEQUEST search program (Eng et al., 1994, J. Am. Soc. Mass Spectrom. 5:976-989) and the method described in PCT/GB01/04034. The first group consists of MSPIs that are decreased in the CSF of subjects having MS as compared with the CSF of subjects free from MS, where the differential presence is significant. The amino acid sequences of tryptic digest peptides of these MSPIs identified by tandem mass spectrometry and database searching as described in the Examples, infra are listed in Table IV in addition to the pIs and MWs of these MSPIs.

Table IV. MSPIs Decreased in CSF of Subjects Having MS

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
MSF-1	MSPI-1	8.75	21021	APEAQVSVQPNFQQDK	SEQ ID NO.: 25
				TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-2	MSPI-2	5.62	34394	FMETVAEK	SEQ ID NO.: 110
				ELDESLQVAER	SEQ ID NO.: 83
MSF-3	MSPI-3	9.75	11627	LVGGPMDASVEEEGVR	SEQ ID NO.: 211
MSF-4	MSPI-4	6.24	102603	AASGTQNNVLR	SEQ ID NO.: 3
				EQTMSECEAGALR	SEQ ID NO.: 94
MSF-5	MSPI-5.1	6.18	105482	AASGTQNNVLR	SEQ ID NO.: 3
				GCPTEEGCGER	SEQ ID NO.: 120
MSF-6	MSPI-6	5.91	21888	AQGFTEDTIVFLPQTDK	SEQ ID NO.: 26
MSF-7	MSPI-7	5.52	56560	VLSALQAVQGLLVAQGR	SEQ ID NO.: 338
				FMQAVTGWK	SEQ ID NO.: 111
				LQAILGVPWK	SEQ ID NO.: 204

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
				DPTFIPAPIQAK	SEQ ID NO.: 62
				SLDFTELDVAAEK	SEQ ID NO.: 276
				ALQDQLVLVAAK	SEQ ID NO.: 22
MSF-8	MSPI-8.1	5.49	34394	ASSIIDELFQDR	SEQ ID NO.: 28
MSF-8	MSPI-8.2	5.49	34394	LEEQAQQIR	SEQ ID NO.: 183
				QQTEWQSGQR	SEQ ID NO.: 251
				LGPLVEQGR	SEQ ID NO.: 192
				GEVQAMLGQSTEELR	SEQ ID NO.: 123
				SELEEQLTPVAEETR	SEQ ID NO.: 267
MSF-9	MSPI-9	6.01	184426	VGFYESDVMGR	SEQ ID NO.: 333
				LPPNVVEESAR	SEQ ID NO.: 202
				VTAAPQSVCALR	SEQ ID NO.: 346
				LVHVEEPHTETVR	SEQ ID NO.: 212
MSF-10	MSPI-10	6.06	184426	VGFYESDVMGR	SEQ ID NO.: 333
				LPPNVVEESAR	SEQ ID NO.: 202
				AIGYLNTGYQR	SEQ ID NO.: 17
MSF-11	MSPI-11	5.96	185225	VGFYESDVMGR	SEQ ID NO.: 333
				VTAAPQSVCALR	SEQ ID NO.: 346
				AIGYLNTGYQR	SEQ ID NO.: 17
MSF-12	MSPI-12.1	5.41	51566	LAAAVSNFGYDLYR	SEQ ID NO.: 176
				TSLEDFYLDEER	SEQ ID NO.: 312
MSF-12	MSPI-12.2	5.41	51566	YEAAVPDPR	SEQ ID NO.: 366
				TALASGGVLDASGDYR	SEQ ID NO.: 287
				EPGEFALLR	SEQ ID NO.: 89
MSF-13	MSPI-13	5.92	185225	LPPNVVEESAR	SEQ ID NO.: 202
				AIGYLNTGYQR	SEQ ID NO.: 17
				HYDGSYSTFGER	SEQ ID NO.: 150
MSF-14	MSPI-14	6.80	32080	VVEEQESR	SEQ ID NO.: 349
				VHYTVCIWR	SEQ ID NO.: 334
				CSVFYGAPSK	SEQ ID NO.: 47
				ITQVLHFTK	SEQ ID NO.: 165
				VEYGFQVK	SEQ ID NO.: 329
				FACYYPR	SEQ ID NO.: 102
				GLQDEDGYR	SEQ ID NO.: 133
MSF-15	MSPI-15	5.88	186027	LPPNVVEESAR	SEQ ID NO.: 202
MSF-16	MSPI-16	6.14	186027	VGFYESDVMGR	SEQ ID NO.: 333
				LPPNVVEESAR	SEQ ID NO.: 202
MSF-18	MSPI-18	5.79	186832	LPPNVVEESAR	SEQ ID NO.: 202
				VGFYESDVMGR	SEQ ID NO.: 333
				AIGYLNTGYQR	SEQ ID NO.: 17
MSF-20	MSPI-20	5.50	20607	QITVNDLPVGR	SEQ ID NO.: 246
MSF-21	MSPI-21	4.70	43626	AYLEEECPATLR	SEQ ID NO.: 35
MSF-22	MSPI-22	5.75	186832	LPPNVVEESAR	SEQ ID NO.: 202
				AIGYLNTGYQR	SEQ ID NO.: 17
MSF-24	MSPI-24	6.18	187641	LPPNVVEESAR	SEQ ID NO.: 202
MSF-25	MSPI-25	6.23	48975	YDSVSVFNGAVSDDSR	SEQ ID NO.: 365
				GVSYLLMGQVEENR	SEQ ID NO.: 142
MSF-27	MSPI-27	6.11	126160	TLNICEVGTIR	SEQ ID NO.: 309
				QLEWGLER	SEQ ID NO.: 247
MSF-28	MSPI-28	6.23	186832	LPPNVVEESAR	SEQ ID NO.: 202
MSF-29	MSPI-29	6.10	184426	AIGYLNTGYQR	SEQ ID NO.: 17

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
				LPPNVVEESAR	SEQ ID NO.: 202
MSF-30	MSPI-30	7.82	31104	GLQDEDGYR	SEQ ID NO.: 133
MSF-31	MSPI-31	5.84	186832	VGFYESDVMGR	SEQ ID NO.: 333
				AIGYLNTGYQR	SEQ ID NO.: 17
MSF-32	MSPI-32	6.03	135312	HEGSFIQGAEK	SEQ ID NO.: 145
				TLNICEVGTIR	SEQ ID NO.: 309
				QLEWGLER	SEQ ID NO.: 247
MSF-33	MSPI-33.1	4.89	52993	YEAAVPDPR	SEQ ID NO.: 366
				EPGEFALLR	SEQ ID NO.: 89
MSF-33	MSPI-33.2	4.89	52993	SGNENGEFYLR	SEQ ID NO.: 269
MSF-34	MSPI-34	5.59	21103	QITVNDLPVGR	SEQ ID NO.: 246
MSF-36	MSPI-36	5.93	49874	YEAAVPDPR	SEQ ID NO.: 366
				EPGEFALLR	SEQ ID NO.: 89
				TALASGGVLDASGDYR	SEQ ID NO.: 287
MSF-37	MSPI-37	5.30	179707	QSEDSTFYLGER	SEQ ID NO.: 252
				NNEGTYYSPNYNPQSR	SEQ ID NO.: 232
				GAYPLSIEPIGVR	SEQ ID NO.: 119
MSF-39	MSPI-39.1	4.82	98505	TGYYFDGISR	SEQ ID NO.: 301
				CLAFECPENYR	SEQ ID NO.: 42
				IIEVEEEQEDPYLNDR	SEQ ID NO.: 159
MSF-39	MSPI-39.2	4.82	98505	SILFVPTSAPR	SEQ ID NO.: 274
				FAFQAEVNR	SEQ ID NO.: 103
				LGVIEDHSNR	SEQ ID NO.: 193
				NLLHVTDTGVGMTR	SEQ ID NO.: 230
				EEEAIQLDGLNASQIR	SEQ ID NO.: 71
MSF-40	MSPI-40	5.35	11955	LVGGPMDASVEEEGVR	SEQ ID NO.: 211
MSF-42	MSPI-42.2	5.98	64540	VWVYPPEK	SEQ ID NO.: 351
				NFPSPVDAAFR	SEQ ID NO.: 225
				GECQAEGVLFFQGDR	SEQ ID NO.: 122
				DYFMPCPGR	SEQ ID NO.: 67
MSF-43	MSPI-43	5.54	37852	IDVHWTR	SEQ ID NO.: 153
				AYLEEECPATLR	SEQ ID NO.: 35
				AGEVQEPELR	SEQ ID NO.: 16
				DYIEFNK	SEQ ID NO.: 68
				AREDIFMETLK	SEQ ID NO.: 27
				WEAEPVYVQR	SEQ ID NO.: 354
MSF-44	MSPI-44	6.28	27735	AVVVHAGEDDLGR	SEQ ID NO.: 34
				GGNQASVENGNAGR	SEQ ID NO.: 126
				VTEIWQEVMQR	SEQ ID NO.: 347
MSF-47	MSPI-47	5.49	64150	VWVYPPEK	SEQ ID NO.: 351
				GECQAEGVLFFQGDR	SEQ ID NO.: 122
				NFPSPVDAAFR	SEQ ID NO.: 225
				DYFMPCPGR	SEQ ID NO.: 67
MSF-48	MSPI-48.1	4.77	99610	TGYYFDGISR	SEQ ID NO.: 301
				CLAFECPENYR	SEQ ID NO.: 42
				IIEVEEEQEDPYLNDR	SEQ ID NO.: 159
MSF-48	MSPI-48.2	4.77	99610	SILFVPTSAPR	SEQ ID NO.: 274
				FAFQAEVNR	SEQ ID NO.: 103
				LGVIEDHSNR	SEQ ID NO.: 193
				NLLHVTDTGVGMTR	SEQ ID NO.: 230
				EEEAIQLDGLNASQIR	SEQ ID NO.: 71

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
MSF-48	MSPI-48.3	4.77	99610	SILFVPTSAPR	SEQ ID NO.: 274
				FAFQAEVNR	SEQ ID NO.: 103
				EEEAIQLDGLNASQIR	SEQ ID NO.: 71
MSF-49	MSPI-49.1	4.54	74941	MNPLEQYER	SEQ ID NO.: 217
				VIALINDQR	SEQ ID NO.: 335
MSF-49	MSPI-49.2	4.54	74941	SGNENGEFYLR	SEQ ID NO.: 269
MSF-51	MSPI-51.1	7.48	11668	LVGGPMDASVEEEGVR	SEQ ID NO.: 211
MSF-52	MSPI-52.1	5.72	146050	GYTQQLAFR	SEQ ID NO.: 144
				AVLYNYR	SEQ ID NO.: 33
				SGSDEVQVGQQR	SEQ ID NO.: 270
				AGDFLEANYMNLQR	SEQ ID NO.: 14
MSF-52	MSPI-52.2	5.72	146050	GYTQQLAFR	SEQ ID NO.: 144
				AVLYNYR	SEQ ID NO.: 33
				SGSDEVQVGQQR	SEQ ID NO.: 270
MSF-54	MSPI-54	8.64	20604	AQGFTEDTIVFLPQTDK	SEQ ID NO.: 26
				TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-55	MSPI-55	5.65	150534	VQVTSQEYSAR	SEQ ID NO.: 342
MSF-56	MSPI-56	5.49	158545	GAYPLSIEPIGVR	SEQ ID NO.: 119
				QSEDSTFYLGER	SEQ ID NO.: 252
				NNEGTYYSPNYNPQSR	SEQ ID NO.: 232
MSF-58	MSPI-58	5.66	186832	LPPNVVEESAR	SEQ ID NO.: 202
MSF-60	MSPI-60	5.60	150534	IVESYQIR	SEQ ID NO.: 167
				NDGGIYTCFAENNR	SEQ ID NO.: 222
				TILSDDWK	SEQ ID NO.: 305
				FIPLIPLPER	SEQ ID NO.: 106
MSF-62	MSPI-62.2	6.52	175109	NGFYPATR	SEQ ID NO.: 226
				EIMENYNIALR	SEQ ID NO.: 79
MSF-63	MSPI-63	5.00	171366	VEEVKPLEGR	SEQ ID NO.: 325
				SMEQNGPGLEYR	SEQ ID NO.: 277
				VEEVKPLEGR	SEQ ID NO.: 325
				SMEQNGPGLEYR	SEQ ID NO.: 277
MSF-64	MSPI-64.1	6.32	178932	TGDEITYQCR	SEQ ID NO.: 300
				NGFYPATR	SEQ ID NO.: 226
MSF-65	MSPI-65	6.18	136566	QLEWGLER	SEQ ID NO.: 247
				GFVVAGPSR	SEQ ID NO.: 125
MSF-66	MSPI-66	5.49	98505	QGSFQGGFR	SEQ ID NO.: 244
MSF-67	MSPI-67.1	5.16	53159	TALASGGVLDASGDYR	SEQ ID NO.: 287
				WVNLPEESLLR	SEQ ID NO.: 363
MSF-67	MSPI-67.2	5.16	53159	LAAAVSNFGYDLYR	SEQ ID NO.: 176
				KTSLEDFYLDEER	SEQ ID NO.: 173
				TSLEDFYLDEER	SEQ ID NO.: 312
				ALYYDLISSPDIHGTYK	SEQ ID NO.: 24
MSF-69	MSPI-69	4.51	126852	GLGEISAASEFK	SEQ ID NO.: 131
MSF-70	MSPI-70	6.15	89080	QDACQGDSGGVFAVR	SEQ ID NO.: 238
MSF-72	MSPI-72	5.09	156672	ALYLQYTDETFR	SEQ ID NO.: 23
				NNEGTYYSPNYNPQSR	SEQ ID NO.: 232
				QSEDSTFYLGER	SEQ ID NO.: 252
				GAYPLSIEPIGVR	SEQ ID NO.: 119
				YTVNQCR	SEQ ID NO.: 375
MSF-73	MSPI-73	7.26	33226	DNLAIQTR	SEQ ID NO.: 61
				ITVVDALHEIPVK	SEQ ID NO.: 166

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
				TELLPGDR	SEQ ID NO.: 294
MSF-75	MSPI-75	10.06	11587	LVGGPMDASVEEEGVR	SEQ ID NO.: 211
				ALDFAVGEYNK	SEQ ID NO.: 19
MSF-76	MSPI-76	5.72	151186	GYTQQLAFR	SEQ ID NO.: 144
				AVLYNYR	SEQ ID NO.: 33
				SGSDEVQVGQQR	SEQ ID NO.: 270
				AGDFLEANYMNLQR	SEQ ID NO.: 14
MSF-77	MSPI-77.2	6.38	177393	IDVHLVPDR	SEQ ID NO.: 152
				EYHFGQAVR	SEQ ID NO.: 100
MSF-78	MSPI-78.1	6.25	33313	ASSIIDELFQDR	SEQ ID NO.: 28
				TLLSNLEEAK	SEQ ID NO.: 307
MSF-78	MSPI-78.2	6.25	33313	APEAQVSVQPNFQQDK	SEQ ID NO.: 25
				TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-79	MSPI-79	5.48	85680	NDGGIYTCFAENNR	SEQ ID NO.: 222
MSF-80	MSPI-80.1	6.06	60926	TFTCTAAYPESK	SEQ ID NO.: 297
				QEPSQGTTTFAVTSILR	SEQ ID NO.: 240
				WLQGSQELPR	SEQ ID NO.: 359
MSF-81	MSPI-81	5.94	42899	GSPAINVAVHVFR	SEQ ID NO.: 140
MSF-82	MSPI-82	5.72	100168	MILEIAPTSDNDFGR	SEQ ID NO.: 216
				LTIYNANIEDAGIYR	SEQ ID NO.: 210
				IEIFQTLPVR	SEQ ID NO.: 156
MSF-83	MSPI-83	5.51	44365	IVFLEEASQQEK	SEQ ID NO.: 168
				FQNIDFAEEVYTR	SEQ ID NO.: 114
				FLYLGDDR	SEQ ID NO.: 109
MSF-84	MSPI-84.1	5.53	99610	QGSFQGGFR	SEQ ID NO.: 244
MSF-84	MSPI-84.2	5.53	99610	IEIFQTLPV	SEQ ID NO.: 155
				NIINSDGGPYVCR	SEQ ID NO.: 228
MSF-86	MSPI-86	4.54	27735	LSELIQPLPLER	SEQ ID NO.: 205
				KPNLQVFLGK	SEQ ID NO.: 171
				GLVSWGNIPCGSK	SEQ ID NO.: 135
				YTNWIQK	SEQ ID NO.: 374
MSF-87	MSPI-87	5.29	125687	AFLFQDTPR	SEQ ID NO.: 10
MSF-89	MSPI-89.1	5.58	63762	VWVYPPEK	SEQ ID NO.: 351
				NFPSPVDAAFR	SEQ ID NO.: 225
				DYFMPCPGR	SEQ ID NO.: 67
				RLWWLDLK	SEQ ID NO.: 258
				GECQAEGVLFFQGDR	SEQ ID NO.: 122
MSF-90	MSPI-90	6.08	93159	EPGLQIWR	SEQ ID NO.: 90
				HVVPNEVVVQR	SEQ ID NO.: 149
MSF-91	MSPI-91.1	6.26	21818	VSFLSALEEYTK	SEQ ID NO.: 344
				DEPPQSPWDR	SEQ ID NO.: 56
				WQEEMELYR	SEQ ID NO.: 360
				AKPALEDLR	SEQ ID NO.: 18
				LSPLGEEMR	SEQ ID NO.: 206
				DYVSQFEGSALGK	SEQ ID NO.: 69
				THLAPYSDELR	SEQ ID NO.: 302
				ATEHLSTLSEK	SEQ ID NO.: 29
				VQPYLDDFQK	SEQ ID NO.: 341
				QGLLPVLESFK	SEQ ID NO.: 243
				THLAPYSDELR	SEQ ID NO.: 302
MSF-92	MSPI-92	5.54	150534	NDGGIYTCFAENNR	SEQ ID NO.: 222

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
				TILSDDWK	SEQ ID NO.: 305
				IVESYQIR	SEQ ID NO.: 167
				FIPLIPIPER	SEQ ID NO.: 105
MSF-93	MSPI-93.1	5.70	34998	GEVQAMLGQSTEELR	SEQ ID NO.: 123
				AATVGSLAGQPLQER	SEQ ID NO.: 4
				SELEEQLTPVAEETR	SEQ ID NO.: 267
				LEEQAQQIR	SEQ ID NO.: 183
				LGPLVEQGR	SEQ ID NO.: 192
MSF-93	MSPI-93.2	5.70	34998	ASSIIDELFQDR	SEQ ID NO.: 28
				ELDESLQVAER	SEQ ID NO.: 83
MSF-94	MSPI-94	5.63	178161	CTSTGWIPAPR	SEQ ID NO.: 48
MSF-95	MSPI-95	5.28	52673	IPIEDGSGEVVLSR	SEQ ID NO.: 162
				VLLDGVQNPR	SEQ ID NO.: 337
				TIYTPGSTVLYR	SEQ ID NO.: 306
				QELSEAEQATR	SEQ ID NO.: 239
MSF-96	MSPI-96	5.80	91613	TGAQELLR	SEQ ID NO.: 299
				HVVPNEVVVQR	SEQ ID NO.: 149
MSF-97	MSPI-97	5.71	91103	VELEDWNGR	SEQ ID NO.: 326
				YLQEIYNSNNQK	SEQ ID NO.: 371
MSF-98	MSPI-98	5.54	48679	IGAEVYHNLK	SEQ ID NO.: 158
				LAQANGWGVMVSHR	SEQ ID NO.: 178
				VNQIGSVTESIQACK	SEQ ID NO.: 340
				VVIGMDVAASEFFR	SEQ ID NO.: 350
MSF-99	MSPI-99	5.43	112518	TYFEGER	SEQ ID NO.: 317
				NNAHGYFK	SEQ ID NO.: 231
				CVTDPCQADTIR	SEQ ID NO.: 50
				HNGQIWVLENDR	SEQ ID NO.: 146
				YLELESSGHR	SEQ ID NO.: 369
				TCPTCNDFHGLVQK	SEQ ID NO.: 291
				AFLFQESPR	SEQ ID NO.: 11
				LDQCYCER	SEQ ID NO.: 179
MSF-102	MSPI-102.1	5.95	34494	SWFEPLVEDMQR	SEQ ID NO.: 285
				SELEEQLTPVAEETR	SEQ ID NO.: 267
				KVEQAVETEPEPELR	SEQ ID NO.: 174
				LEEQAQQIR	SEQ ID NO.: 183
				LGPLVEQGR	SEQ ID NO.: 192
				GEVQAMLGQSTEELR	SEQ ID NO.: 123
MSF-102	MSPI-102.2	5.95	34494	TLLSNLEEAK	SEQ ID NO.: 307
				ASSIIDELFQDR	SEQ ID NO.: 28
MSF-102	MSPI-102.3	5.95	34494	LIAPVAEEEATVPNNK	SEQ ID NO.: 194
				LKDDEVAQLK	SEQ ID NO.: 196
				SLADELALVDVLEDK	SEQ ID NO.: 275
				IVVVTAGVR	SEQ ID NO.: 169
				SADTLWDIQK	SEQ ID NO.: 263
				MVVESAYEVIK	SEQ ID NO.: 219
				GLTSVINQK	SEQ ID NO.: 134
MSF-104	MSPI-104	6.29	100168	FLCTGGVSPYADPNTCR	SEQ ID NO.: 107
				GDSGGPLIVHK	SEQ ID NO.: 121
				ALFVSEEEK	SEQ ID NO.: 20
				QLNEINYEDHK	SEQ ID NO.: 248
				LEDSVTYHCSR	SEQ ID NO.: 182

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
MSF-105	MSPI-105.1	4.84	25658	TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
				AQGFTEDTIVFLPQTDK	SEQ ID NO.: 26
MSF-105	MSPI-105.2	4.84	25658	TGAQELLR	SEQ ID NO.: 299
MSF-106	MSPI-106	8.13	68368	IPIEDGSGEVVLSR	SEQ ID NO.: 162
				AELQCPQPAAR	SEQ ID NO.: 7
				KQELSEAEQATR	SEQ ID NO.: 172
				QELSEAEQATR	SEQ ID NO.: 239
MSF-107	MSPI-107	4.95	52196	TALASGGVLDASGDYR	SEQ ID NO.: 287
MSF-108	MSPI-108	6.05	47450	ELLDTVTAPQK	SEQ ID NO.: 84
				TVQAVLTVPK	SEQ ID NO.: 316
				SSFVAPLEK	SEQ ID NO.: 284
				LSYEGEVTK	SEQ ID NO.: 208
				TSLEDFYLDEER	SEQ ID NO.: 312
				LAAAVSNFGYDLYR	SEQ ID NO.: 176
				DTDTGALLFIGK	SEQ ID NO.: 65
MSF-110	MSPI-110	6.17	93159	AGALNSNDAFVLK	SEQ ID NO.: 13
				YIETDPANR	SEQ ID NO.: 368
				HVVPNEVVVQR	SEQ ID NO.: 149
				EPGLQIWR	SEQ ID NO.: 90
MSF-111	MSPI-111	5.67	48092	LAAAVSNFGYDLYR	SEQ ID NO.: 176
				SSFVAPLEK	SEQ ID NO.: 284
				TSLEDFYLDEER	SEQ ID NO.: 312
MSF-112	MSPI-112	5.48	55124	ATVVYQGER	SEQ ID NO.: 30
				EHSSLAFWK	SEQ ID NO.: 77
MSF-113	MSPI-113	6.12	101661	LPPTTTCQQQK	SEQ ID NO.: 203
				GDSGGPLIVHK	SEQ ID NO.: 121
MSF-114	MSPI-114	5.28	154488	ALYLQYTDETFR	SEQ ID NO.: 23
				GAYPLSIEPIGVR	SEQ ID NO.: 119
				YTVNQCR	SEQ ID NO.: 375
				QSEDSTFYLGER	SEQ ID NO.: 252
MSF-116	MSPI-116	5.39	142313	AETYEGVYQCTAR	SEQ ID NO.: 9
				GKPPPSFSWTR	SEQ ID NO.: 128
				ERPPTFLTPEGNASNK	SEQ ID NO.: 95
				IDGDTIIFSNVQER	SEQ ID NO.: 151
				NALGAIHHTISVR	SEQ ID NO.: 220
				NEVHLEIK	SEQ ID NO.: 223
				QPEYAVVQR	SEQ ID NO.: 250
MSF-117	MSPI-117	5.00	132839	LICSELNGR	SEQ ID NO.: 195
				EGLDLQVLEDSGR	SEQ ID NO.: 75
				GNLAGLTLR	SEQ ID NO.: 137
MSF-119	MSPI-119	6.00	49723	TALASGGVLDASGDYR	SEQ ID NO.: 287
MSF-120	MSPI-120	5.78	72071	HVVPNEVVVQR	SEQ ID NO.: 149
				TGAQELLR	SEQ ID NO.: 299
MSF-122	MSPI-122	5.03	17230	MPCAELVR	SEQ ID NO.: 218
				LEGEACGVYTPR	SEQ ID NO.: 184
				EPGCGCCSVCAR	SEQ ID NO.: 88
MSF-123	MSPI-123.1	5.89	91613	EVQGFESATFLGYFK	SEQ ID NO.: 98
				HVVPNEVVVQR	SEQ ID NO.: 149
				RTPITVVK	SEQ ID NO.: 260
				EPGLQIWR	SEQ ID NO.: 90
MSF-125	MSPI-125.1	4.46	29058	AVVVHAGEDDLGR	SEQ ID NO.: 34

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
MSF-125	MSPI-125.2	4.46	29058	GATADPLCAPAR	SEQ ID NO.: 118
				TALAVDGEAR	SEQ ID NO.: 288
				LGERPPALLGSQGLR	SEQ ID NO.: 191
MSF-126	MSPI-126.1	5.86	17929	GLEEELQFSLGSK	SEQ ID NO.: 129
MSF-126	MSPI-126.2	5.86	17929	EVAGLWIK	SEQ ID NO.: 96
				EGTYSLPK	SEQ ID NO.: 76
MSF-127	MSPI-127.1	5.62	54294	VLSALQAVQGLLVAQGR	SEQ ID NO.: 338
				DPTFIPAPIQAK	SEQ ID NO.: 62
				SLDFTELDVAAEK	SEQ ID NO.: 276
				ALQDQLVLVAAK	SEQ ID NO.: 22
MSF-128	MSPI-128	5.57	142313	GYTQQLAFR	SEQ ID NO.: 144
				AVLYNYR	SEQ ID NO.: 33
				SGSDEVQVGQQR	SEQ ID NO.: 270
				SNLDEDIIAEENIVSR	SEQ ID NO.: 279
MSF-130	MSPI-130	4.88	95795	GYHLNEEGTR	SEQ ID NO.: 143
				CLAFECPENYR	SEQ ID NO.: 42
				TGYYFDGISR	SEQ ID NO.: 301
MSF-131	MSPI-131	5.03	50944	GTQEQDFYVTSETVVR	SEQ ID NO.: 141
				EDQYHYLLDR	SEQ ID NO.: 70
				TLYLADTFPTNFR	SEQ ID NO.: 310
MSF-132	MSPI-132	5.16	41028	ITCTEEGWSPTPK	SEQ ID NO.: 164
				INHGILYDEEK	SEQ ID NO.: 161
				EIMENYNIALR	SEQ ID NO.: 79
MSF-133	MSPI-133	5.87	67575	AADDTWEPFASGK	SEQ ID NO.: 1
				GSPAINVAVHVFR	SEQ ID NO.: 140
MSF-134	MSPI-134	5.98	90092	HVVPNEVVVQR	SEQ ID NO.: 149
				EPGLQIWR	SEQ ID NO.: 90
				YIETDPANR	SEQ ID NO.: 368
MSF-135	MSPI-135	5.18	180485	VIAVNEVGR	SEQ ID NO.: 336
MSF-136	MSPI-136	4.70	37742	IETALTSLHQR	SEQ ID NO.: 157
				WTFEACR	SEQ ID NO.: 362
				FQLTFPLR	SEQ ID NO.: 113
				LENLEQYSR	SEQ ID NO.: 186
MSF-137	MSPI-137	5.97	56994	SLDFTELDVAAEK	SEQ ID NO.: 276
				ALQDQLVLVAAK	SEQ ID NO.: 22
				FMQAVTGWK	SEQ ID NO.: 111
				VLSALQAVQGLLVAQGR	SEQ ID NO.: 338
				LQAILGVPWK	SEQ ID NO.: 204
				DPTFIPAPIQAK	SEQ ID NO.: 62
MSF-139	MSPI-139	6.06	28850	VTEIWQEVMQR	SEQ ID NO.: 347
MSF-140	MSPI-140	6.95	19478	EQQALQTVCLK	SEQ ID NO.: 92
				TFHEASEDCISR	SEQ ID NO.: 296
				NWETEITAQPDGGK	SEQ ID NO.: 235
MSF-141	MSPI-141.1	5.77	52354	YTFELSR	SEQ ID NO.: 373
MSF-141	MSPI-141.2	5.77	52354	EPGEFALLR	SEQ ID NO.: 89
				VAMHLVCPSR	SEQ ID NO.: 320
				TALASGGVLDASGDYR	SEQ ID NO.: 287
				YEAAVPDPR	SEQ ID NO.: 366
MSF-142	MSPI-142	5.77	54791	DPTFIPAPIQAK	SEQ ID NO.: 62
				FMQAVTGWK	SEQ ID NO.: 111
				VLSALQAVQGLLVAQGR	SEQ ID NO.: 338

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
				ALQDQLVLVAAK	SEQ ID NO.: 22
				SLDFTELDVAAEK	SEQ ID NO.: 276
				LQAILGVPWK	SEQ ID NO.: 204
MSF-143	MSPI-143.1	5.95	19673	QITVNDLPVGR	SEQ ID NO.: 246
MSF-144	MSPI-144	5.48	46191	YVGGQEHFAHLLILR	SEQ ID NO.: 376
				EQLGEFYEALDCLR	SEQ ID NO.: 91
				TLMFGSYLDDEK	SEQ ID NO.: 308
				NWGLSVYADKPETTK	SEQ ID NO.: 236
				TEDTIFLR	SEQ ID NO.: 292
				TYMLAFDVNDEK	SEQ ID NO.: 318
				WFYIASAFR	SEQ ID NO.: 358
MSF-145	MSPI-145.2	5.40	151841	ALYLQYTDETFR	SEQ ID NO.: 23
				IYHSHIDAPK	SEQ ID NO.: 170
				DLYSGLIGPLIVCR	SEQ ID NO.: 59
				GAYPLSIEPIGVR	SEQ ID NO.: 119
				QSEDSTFYLGER	SEQ ID NO.: 252
MSF-147	MSPI-147.2	7.10	73878	QELSEAEQATR	SEQ ID NO.: 239
				IPIEDGSGEVVLSR	SEQ ID NO.: 162
MSF-148	MSPI-148	5.07	131619	SPEQQETVLDGNLIIR	SEQ ID NO.: 281
				ILDDLSPR	SEQ ID NO.: 160
				LALDNGGLAR	SEQ ID NO.: 177
MSF-149	MSPI-149	6.21	101661	LEDSVTYHCSR	SEQ ID NO.: 182
				GDSGGPLIVHK	SEQ ID NO.: 121
				LPPTTTCQQQK	SEQ ID NO.: 203
				CLVNLIEK	SEQ ID NO.: 45
				DAQYAPGYDK	SEQ ID NO.: 51
MSF-150	MSPI-150	5.50	151186	VQVTSQEYSAR	SEQ ID NO.: 342
MSF-151	MSPI-151	9.39	11427	ALDFAVGEYNK	SEQ ID NO.: 19
				LVGGPMDASVEEEGVR	SEQ ID NO.: 211
MSF-152	MSPI-152	5.55	60192	WLQGSQELPR	SEQ ID NO.: 359
				DASGVTFTWPSTSGK	SEQ ID NO.: 52
				TFTCTAAYPESK	SEQ ID NO.: 297
MSF-154	MSPI-154.1	5.28	142929	QSEDSTFYLGER	SEQ ID NO.: 252
				ALYLQYTDETFR	SEQ ID NO.: 23
				GAYPLSIEPIGVR	SEQ ID NO.: 119
				NNEGTYYSPNYNPQSR	SEQ ID NO.: 232
MSF-154	MSPI-154.2	5.28	142929	QPEYAVVQR	SEQ ID NO.: 250
MSF-154	MSPI-154.3	5.28	142929	AFLFQDTPR	SEQ ID NO.: 10
MSF-155	MSPI-155.1	6.33	49273	YLQEIYNSNNQK	SEQ ID NO.: 371
				DTVQIHDITGK	SEQ ID NO.: 66
				DNCCILDER	SEQ ID NO.: 60
				LTIGEGQQHHLGGAK	SEQ ID NO.: 209
				VELEDWNGR	SEQ ID NO.: 326
				YEASILTHDSSIR	SEQ ID NO.: 367
MSF-155	MSPI-155.2	6.33	49273	TSLEDFYLDEER	SEQ ID NO.: 312
				LAAAVSNFGYDLYR	SEQ ID NO.: 176
MSF-155	MSPI-155.3	6.33	49273	EPGEFALLR	SEQ ID NO.: 89
				YEAAVPDPR	SEQ ID NO.: 366
				TALASGGVLDASGDYR	SEQ ID NO.: 287
MSF-156	MSPI-156.2	5.85	48238	ELLDTVTAPQK	SEQ ID NO.: 84
				TVQAVLTVPK	SEQ ID NO.: 316

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
				LSYEGEVTK	SEQ ID NO.: 208
				SSFVAPLEK	SEQ ID NO.: 284
				TSLEDFYLDEER	SEQ ID NO.: 312
				LAAAVSNFGYDLYR	SEQ ID NO.: 176
				DTDTGALLFIGK	SEQ ID NO.: 65
MSF-158	MSPI-158.2	5.88	64150	YYCFQGNQFLR	SEQ ID NO.: 380
				GECQAEGVLFFQGDR	SEQ ID NO.: 122
				VWVYPPEK	SEQ ID NO.: 351
				DYFMPCPGR	SEQ ID NO.: 67
				NFPSPVDAAFR	SEQ ID NO.: 225
				RLWWLDLK	SEQ ID NO.: 258
MSF-159	MSPI-159	6.37	101661	LPPTTTCQQQK	SEQ ID NO.: 203
				LEDSVTYHCSR	SEQ ID NO.: 182
				QLNEINYEDHK	SEQ ID NO.: 248
				CLVNLIEK	SEQ ID NO.: 45
				VASYGVKPR	SEQ ID NO.: 321
				ALFVSEEEK	SEQ ID NO.: 20
				GDSGGPLIVHK	SEQ ID NO.: 121
MSF-160	MSPI-160	5.54	47450	LAAAVSNFGYDLYR	SEQ ID NO.: 176
				TSLEDFYLDEER	SEQ ID NO.: 312
				SSFVAPLEK	SEQ ID NO.: 284
MSF-161	MSPI-161	6.25	55124	LQAILGVPWK	SEQ ID NO.: 204
				DPTFIPAPIQAK	SEQ ID NO.: 62
				FMQAVTGWK	SEQ ID NO.: 111
				VLSALQAVQGLLVAQGR	SEQ ID NO.: 338
				ALQDQLVLVAAK	SEQ ID NO.: 22
				SLDFTELDVAAEK	SEQ ID NO.: 276
MSF-163	MSPI-163	5.32	142929	QPEYAVVQR	SEQ ID NO.: 250
MSF-165	MSPI-165.1	5.77	50178	DNCCILDER	SEQ ID NO.: 60
				VELEDWNGR	SEQ ID NO.: 326
MSF-165	MSPI-165.2	5.77	50178	YEAAVPDPR	SEQ ID NO.: 366
				VAMHLVCPSR	SEQ ID NO.: 320
				EPGEFALLR	SEQ ID NO.: 89
				TALASGGVLDASGDYR	SEQ ID NO.: 287
MSF-167	MSPI-167	6.17	48679	VWVYPPEK	SEQ ID NO.: 351
				GECQAEGVLFFQGDR	SEQ ID NO.: 122
				DYFMPCPGR	SEQ ID NO.: 67
MSF-168	MSPI-168	5.36	98505	SHALQLNNR	SEQ ID NO.: 272
				QGSFQGGFR	SEQ ID NO.: 244
				EELVYELNPLDHR	SEQ ID NO.: 72
MSF-169	MSPI-169.1	5.12	139109	ILDDLSPR	SEQ ID NO.: 160
				VRPQQLVK	SEQ ID NO.: 343
				FAHTVVTSR	SEQ ID NO.: 104
				LALDNGGLAR	SEQ ID NO.: 177
MSF-169	MSPI-169.2	5.12	139109	EYTDASFTNR	SEQ ID NO.: 101
				QYTDSTFR	SEQ ID NO.: 256
				YTVNQCR	SEQ ID NO.: 375
				GAYPLSIEPIGVR	SEQ ID NO.: 119
				QSEDSTFYLGER	SEQ ID NO.: 252
MSF-170	MSPI-170	5.31	156503	NNEGTYYSPNYNPQSR	SEQ ID NO.: 232
				QSEDSTFYLGER	SEQ ID NO.: 252

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
				GAYPLSIEPIGVR	SEQ ID NO.: 119
				ALYLQYTDETFR	SEQ ID NO.: 23
				IYHSHIDAPK	SEQ ID NO.: 170
MSF-171	MSPI-171	4.99	15072	SELEEQLTPVAEETR	SEQ ID NO.: 267
				GEVQAMLGQSTEELR	SEQ ID NO.: 123
				KVEQAVETEPEPELR	SEQ ID NO.: 174
MSF-172	MSPI-172	5.56	54294	IPIEDGSGEVVLSR	SEQ ID NO.: 162
				SGIPIVTSPYQIHFTK	SEQ ID NO.: 268
				TVMVNIENPEGIPVK	SEQ ID NO.: 315
				QELSEAEQATR	SEQ ID NO.: 239
				VLLDGVQNPR	SEQ ID NO.: 337
				GLEVTITAR	SEQ ID NO.: 130
				FYYIYNEK	SEQ ID NO.: 117
				TIYTPGSTVLYR	SEQ ID NO.: 306
MSF-173	MSPI-173.2	5.85	62512	GECQAEGVLFFQGDR	SEQ ID NO.: 122
				NFPSPVDAAFR	SEQ ID NO.: 225
				VWVYPPEK	SEQ ID NO.: 351
				DYFMPCPGR	SEQ ID NO.: 67
MSF-175	MSPI-175	4.98	100168	TGYYFDGISR	SEQ ID NO.: 301
				IIEVEEEQEDPYLNDR	SEQ ID NO.: 159
MSF-176	MSPI-176.1	5.43	48238	LSYEGEVTK	SEQ ID NO.: 208
				SSFVAPLEK	SEQ ID NO.: 284
				LAAAVSNFGYDLYR	SEQ ID NO.: 176
				TSLEDFYLDEER	SEQ ID NO.: 312
				ELLDTVTAPQK	SEQ ID NO.: 84
MSF-176	MSPI-176.2	5.43	48238	QTVPAQILLLHYR	SEQ ID NO.: 253
				EPGEFALLR	SEQ ID NO.: 89
MSF-177	MSPI-177	7.67	68368	SGIPIVTSPYQIHFTK	SEQ ID NO.: 268
				EVVADSVWADVR	SEQ ID NO.: 99
				GLEVTITAR	SEQ ID NO.: 130
				YYTYLIMNK	SEQ ID NO.: 381
				TIYTPGSTVLYR	SEQ ID NO.: 306
				FYYIYNEK	SEQ ID NO.: 117
				IPIEDGSGEVVLSR	SEQ ID NO.: 162
				VLLDGVQNPR	SEQ ID NO.: 337
MSF-178	MSPI-178	8.47	22644	APEAQVSVQPNFQQDK	SEQ ID NO.: 25
				AQGFTEDTIVFLPQTDK	SEQ ID NO.: 26
				TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-179	MSPI-179.1	5.60	46036	SAVQGPPER	SEQ ID NO.: 264
				QEPSQGTTTFAVTSILR	SEQ ID NO.: 240
				WLQGSQELPR	SEQ ID NO.: 359
				YLTWASR	SEQ ID NO.: 372
				DASGVTFTWTPSSGK	SEQ ID NO.: 53
MSF-179	MSPI-179.2	5.60	46036	DYFMPCPGR	SEQ ID NO.: 67
				NFPSPVDAAFR	SEQ ID NO.: 225
				VWVYPPEK	SEQ ID NO.: 351
				GECQAEGVLFFQGDR	SEQ ID NO.: 122
MSF-180	MSPI-180	6.21	36134	DCSGVSLHLTR	SEQ ID NO.: 54
				CVFTGEGR	SEQ ID NO.: 49
MSF-181	MSPI-181.1	5.13	21638	EVAGLWIK	SEQ ID NO.: 96
				EGTYSLPK	SEQ ID NO.: 76

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences f Tryptic Digest Peptides	SEQ ID NO:
MSF-181	MSPI-181.2	5.13	21638	AQGFTEDTIVFLPQTDK	SEQ ID NO.: 26
				TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-182	MSPI-182	5.62	52038	LQAILGVPWK	SEQ ID NO.: 204
				FMQAVTGWK	SEQ ID NO.: 111
				ALQDQLVLVAAK	SEQ ID NO.: 22
				VLSALQAVQGLLVAQGR	SEQ ID NO.: 338
				SLDFTELDVAAEK	SEQ ID NO.: 276
				DPTFIPAPIQAK	SEQ ID NO.: 62
MSF-183	MSPI-183	8.76	18350	TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-184	MSPI-184	6.30	50790	TSLEDFYLDEER	SEQ ID NO.: 312
				LAAAVSNFGYDLYR	SEQ ID NO.: 176
MSF-185	MSPI-185	4.81	18657	VFSNGADLSGVTEEAPLK	SEQ ID NO.: 331
				FLENEDR	SEQ ID NO.: 108
MSF-186	MSPI-186	6.81	48827	VAMHLVCPSR	SEQ ID NO.: 320
				WVNLPEESLLR	SEQ ID NO.: 363
				TALASGGVLDASGDYR	SEQ ID NO.: 287
				YEAAVPDPR	SEQ ID NO.: 366
				EPGEFALLR	SEQ ID NO.: 89
MSF-187	MSPI-187.1	6.01	29689	ELDESLQVAER	SEQ ID NO.: 83
MSF-187	MSPI-187.2	6.01	29689	AADDTWEPFASGK	SEQ ID NO.: 1
				GSPAINVAVHVFR	SEQ ID NO.: 140
MSF-188	MSPI-188.2	5.59	100168	QGEDAEVVCR	SEQ ID NO.: 242
				SNPPASIHWR	SEQ ID NO.: 280
MSF-189	MSPI-189	5.74	65725	GECQAEGVLFFQGDR	SEQ ID NO.: 122
				VWVYPPEK	SEQ ID NO.: 351
				DYFMPCPGR	SEQ ID NO.: 67
				RLWWLDLK	SEQ ID NO.: 258
				NFPSPVDAAFR	SEQ ID NO.: 225
				YYCFQGNQFLR	SEQ ID NO.: 380
MSF-191	MSPI-191	6.24	87097	EPGLQIWR	SEQ ID NO.: 90
				HVVPNEVVVQR	SEQ ID NO.: 149
MSF-192	MSPI-192.1	5.49	19869	TENCAVLSGAANGK	SEQ ID NO.: 295
				LDTLAQEVALLK	SEQ ID NO.: 180
				NWETEITAQPDGGK	SEQ ID NO.: 235
				TFHEASEDCISR	SEQ ID NO.: 296
				NWETEITAQPDGGK	SEQ ID NO.: 235
				CFLAFTQTK	SEQ ID NO.: 41
				EQQALQTVCLK	SEQ ID NO.: 92
MSF-192	MSPI-192.2	5.49	19869	RVIMERDTYPR	SEQ ID NO.: 261
				LDTSQWPLLLK	SEQ ID NO.: 181
MSF-193	MSPI-193	5.90	23795	APEAQVSVQPNFQQDK	SEQ ID NO.: 25
				AQGFTEDTIVFLPQTDK	SEQ ID NO.: 26
				TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-194	MSPI-194.1	6.91	116746	HSIFTPETNPR	SEQ ID NO.: 148
				CEEDEEFTCR	SEQ ID NO.: 37
MSF-194	MSPI-194.2	6.91	116746	CFFQGDHGFDNK	SEQ ID NO.: 40
				IEDIHLLVER	SEQ ID NO.: 154
				CFELQEAGPPDCR	SEQ ID NO.: 39
MSF-195	MSPI-195.1	5.45	66932	GECQAEGVLFFQGDR	SEQ ID NO.: 122
				VWVYPPEK	SEQ ID NO.: 351
				DYFMPCPGR	SEQ ID NO.: 67

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
				NFPSPVDAAFR	SEQ ID NO.: 225
MSF-196	MSPI-196	8.22	51254	DPTFIPAPIQAK	SEQ ID NO.: 62
				ALQDQLVLVAAK	SEQ ID NO.: 22
				FMQAVTGWK	SEQ ID NO.: 111
				SLDFTELDVAAEK	SEQ ID NO.: 276
				VLSALQAVQGLLVAQGR	SEQ ID NO.: 338
				LQAILGVPWK	SEQ ID NO.: 204
MSF-197	MSPI-197	5.11	50790	LAAAVSNFGYDLYR	SEQ ID NO.: 176
				TSLEDFYLDEER	SEQ ID NO.: 312
MSF-198	MSPI-198	5.59	20201	EQQALQTVCLK	SEQ ID NO.: 92
				LDTLAQEVALLK	SEQ ID NO.: 180
				CFLAFTQTK	SEQ ID NO.: 41
				NWETEITAQPDGGK	SEQ ID NO.: 235
				TFHEASEDCISR	SEQ ID NO.: 296
MSF-199	MSPI-199.1	5.43	49573	ELLDTVTAPQK	SEQ ID NO.: 84
				TVQAVLTVPK	SEQ ID NO.: 316
				ALYYDLISSPDIHGTYK	SEQ ID NO.: 24
				DTDTGALLFIGK	SEQ ID NO.: 65
				LAAAVSNFGYDLYR	SEQ ID NO.: 176
				SSFVAPLEK	SEQ ID NO.: 284
				TSLEDFYLDEER	SEQ ID NO.: 312
				LSYEGEVTK	SEQ ID NO.: 208
MSF-199	MSPI-199.2	5.43	49573	TALASGGVLDASGDYR	SEQ ID NO.: 287
MSF-200	MSPI-200	7.14	116746	EQQCVIMAENR	SEQ ID NO.: 93
				NLDENYCR	SEQ ID NO.: 229
				HSIFTPETNPR	SEQ ID NO.: 148
				WELCDIPR	SEQ ID NO.: 355
				WSSTSPHRPR	SEQ ID NO.: 361
				CEEDEEFTCR	SEQ ID NO.: 37
MSF-201	MSPI-201	6.65	12463	AADDTWEPFASGK	SEQ ID NO.: 1
				GSPAINVAVHVFR	SEQ ID NO.: 140
MSF-202	MSPI-202.1	4.93	81483	SPQELLCGASLISDR	SEQ ID NO.: 282
				ELLESYIDGR	SEQ ID NO.: 85
				TATSEYQTFFNPR	SEQ ID NO.: 289
MSF-202	MSPI-202.2	4.93	81483	WEPDPQR	SEQ ID NO.: 356
				VIALINDQR	SEQ ID NO.: 335
MSF-203	MSPI-203	6.00	38405	AYLEEECPATLR	SEQ ID NO.: 35
				WEAEPVYVQR	SEQ ID NO.: 354
MSF-205	MSPI-205	5.28	72474	AADDTWEPFASGK	SEQ ID NO.: 1
				GSPAINVAVHVFR	SEQ ID NO.: 140
MSF-206	MSPI-206	6.47	28747	NPNLPPETVDSLK	SEQ ID NO.: 233
				IPTTFENGR	SEQ ID NO.: 163
MSF-207	MSPI-207	4.94	134070	EGLDLQVLEDSGR	SEQ ID NO.: 75
				GNLAGLTLR	SEQ ID NO.: 137
				QFPTPGIR	SEQ ID NO.: 241
MSF-208	MSPI-208	5.37	123390	HNGQIWVLENDR	SEQ ID NO.: 146
				AFLFQESPR	SEQ ID NO.: 11
				YLELESSGHR	SEQ ID NO.: 369
				LDQCYCER	SEQ ID NO.: 179
				NNAHGYFK	SEQ ID NO.: 231
				CVTDPCQADTIR	SEQ ID NO.: 50

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
				TCPTCNDFHGLVQK	SEQ ID NO.: 291
MSF-209	MSPI-209	5.49	24374	TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
				AQGFTEDTIVFLPQTDK	SEQ ID NO.: 26
MSF-210	MSPI-210	5.66	50026	TVQAVLTVPK	SEQ ID NO.: 316
				LSYEGEVTK	SEQ ID NO.: 208
				SSFVAPLEK	SEQ ID NO.: 284
				TSLEDFYLDEER	SEQ ID NO.: 312
				LAAAVSNFGYDLYR	SEQ ID NO.: 176
MSF-211	MSPI-211	5.19	50178	SNAQGIDLNR	SEQ ID NO.: 278
				NFPDLDR	SEQ ID NO.: 224
MSF-212	MSPI-212.2	5.79	64150	YYCFQGNQFLR	SEQ ID NO.: 380
				RLWWLDLK	SEQ ID NO.: 258
				VWVYPPEK	SEQ ID NO.: 351
				GECQAEGVLFFQGDR	SEQ ID NO.: 122
				NFPSPVDAAFR	SEQ ID NO.: 225
				DYFMPCPGR	SEQ ID NO.: 67
MSF-213	MSPI-213	5.83	13375	GGSTSYGTGSETESPR	SEQ ID NO.: 127
				ADSGEGDFLAEGGGVR	SEQ ID NO.: 5
				GLIDEVNQDFTNR	SEQ ID NO.: 132
MSF-215	MSPI-215	5.11	35613	ELDESLQVAER	SEQ ID NO.: 83
				ASSIIDELFQDR	SEQ ID NO.: 28
MSF-216	MSPI-216	9.43	68368	VDFTLSSER	SEQ ID NO.: 322
				AEFQDALEK	SEQ ID NO.: 6
				SCGLHQLLR	SEQ ID NO.: 265
				TTNIQGINLLFSSR	SEQ ID NO.: 313
				VGDTLNLNLR	SEQ ID NO.: 332
				LNMGITDLQGLR	SEQ ID NO.: 199
MSF-217	MSPI-217.3	9.04	11790	LVGGPMDASVEEEGVR	SEQ ID NO.: 211
				ALDFAVGEYNK	SEQ ID NO.: 19
MSF-218	MSPI-218	5.08	91613	GYHLNEEGTR	SEQ ID NO.: 143
MSF-219	MSPI-219	7.01	117828	CEEDEEFTCR	SEQ ID NO.: 37
				FVTWIEGVMR	SEQ ID NO.: 116
				HSIFTPETNPR	SEQ ID NO.: 148
				EQQCVIMAENR	SEQ ID NO.: 93
				NLDENYCR	SEQ ID NO.: 229
				WEYCNLK	SEQ ID NO.: 357
MSF-220	MSPI-220	4.56	46503	TSLEDFYLDEER	SEQ ID NO.: 312
				SSFVAPLEK	SEQ ID NO.: 284
				LAAAVSNFGYDLYR	SEQ ID NO.: 176
MSF-221	MSPI-221.1	5.55	66125	GECQAEGVLFFQGDR	SEQ ID NO.: 122
				DYFMPCPGR	SEQ ID NO.: 67
				VWVYPPEK	SEQ ID NO.: 351
				NFPSPVDAAFR	SEQ ID NO.: 225
				YYCFQGNQFLR	SEQ ID NO.: 380
				RLWWLDLK	SEQ ID NO.: 258
				LTIGEGQQHHLGGAK	SEQ ID NO.: 209
				VELEDWNGR	SEQ ID NO.: 326
				RLDGSVDFK	SEQ ID NO.: 257
				YLQEIYNSNNQK	SEQ ID NO.: 371
MSF-222	MSPI-222.2	5.54	49573	LAAAVSNFGYDLYR	SEQ ID NO.: 176
				TSLEDFYLDEER	SEQ ID NO.: 312

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
MSF-223	MSPI-223.1	5.62	50026	TSLEDFYLDEER	SEQ ID NO.: 312
MSF-223	MSPI-223.2	5.62	50026	IETALTSLHQR	SEQ ID NO.: 157
				LENLEQYSR	SEQ ID NO.: 186
				FQLTFPLR	SEQ ID NO.: 113
				LPFVINDGK	SEQ ID NO.: 201
MSF-224	MSPI-224.1	5.07	65130	VEESELAR	SEQ ID NO.: 324
				SIEVFGQFNGK	SEQ ID NO.: 273
				CLCACPFK	SEQ ID NO.: 44
				TEHYEEQIEAFK	SEQ ID NO.: 293
				DGNTLTYYR	SEQ ID NO.: 57
				DRDGNTLTYYR	SEQ ID NO.: 64
				RPWNVASLIYETK	SEQ ID NO.: 259
MSF-225	MSPI-225	4.64	122257	NAPTPQEFR	SEQ ID NO.: 221
				AGEQDATIHLK	SEQ ID NO.: 15
				GLGEISAASEFK	SEQ ID NO.: 131
				DIQVIVNVPPTIQAR	SEQ ID NO.: 58
MSF-226	MSPI-226	4.81	50178	VEQATQAIPMER	SEQ ID NO.: 327
				QMYPELQIAR	SEQ ID NO.: 249
MSF-228	MSPI-228.1	4.95	44515	GECQAEGVLFFQGDR	SEQ ID NO.: 122
				DYFMPCPGR	SEQ ID NO.: 67
				NFPSPVDAAFR	SEQ ID NO.: 225
MSF-228	MSPI-228.2	4.95	44515	NGVAQEPVHLDSPAIK	SEQ ID NO.: 227
				CLAPLEGAR	SEQ ID NO.: 43
				CEGPIPDVTFELLR	SEQ ID NO.: 38
				HQFLLTGDTQGR	SEQ ID NO.: 147
				ATWSGAVLAGR	SEQ ID NO.: 31
MSF-229	MSPI-229.1	6.07	79685	AEMADQAAAWLTR	SEQ ID NO.: 8
MSF-229	MSPI-229.2	6.07	79685	QIQVSWLR	SEQ ID NO.: 245
				VSVFVPPR	SEQ ID NO.: 345
				YVTSAPMPEPQAPGR	SEQ ID NO.: 377
MSF-230	MSPI-230	6.98	97467	EPGLQIWR	SEQ ID NO.: 90
				HVVPNEVVVQR	SEQ ID NO.: 149
				TGAQELLR	SEQ ID NO.: 299
MSF-231	MSPI-231	5.30	49423	VAMHLVCPSR	SEQ ID NO.: 320
				WVNLPEESLLR	SEQ ID NO.: 363
				TALASGGVLDASGDYR	SEQ ID NO.: 287
				YEAAVPDPR	SEQ ID NO.: 366
				EPGEFALLR	SEQ ID NO.: 89
MSF-232	MSPI-232	7.27	27636	IPTTFENGR	SEQ ID NO.: 163
MSF-233	MSPI-233.1	7.35	36450	KYNELLK	SEQ ID NO.: 175
				ASSIIDELFQDR	SEQ ID NO.: 28
				EILSVDCSTNNPSQAK	SEQ ID NO.: 78
				ELDESLQVAER	SEQ ID NO.: 83
MSF-233	MSPI-233.2	7.35	36450	LGADMEDVCGR	SEQ ID NO.: 190
				QWAGLVEK	SEQ ID NO.: 255
				QQTEWQSGQR	SEQ ID NO.: 251
				SWFEPLVEDMQR	SEQ ID NO.: 285
				AATVGSLAGQPLQER	SEQ ID NO.: 4
				GEVQAMLGQSTEELR	SEQ ID NO.: 123
				SELEEQLTPVAEETR	SEQ ID NO.: 267
				LEEQAQQIR	SEQ ID NO.: 183

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
				LGPLVEQGR	SEQ ID NO.: 192
MSF-234	MSPI-234	5.37	24470	TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-235	MSPI-235	5.36	20134	EVDSGNDIYGNPIK	SEQ ID NO.: 97
				SDGSCAWYR	SEQ ID NO.: 266
MSF-236	MSPI-236.1	5.20	72071	LETPDFQLFK	SEQ ID NO.: 187
				CLAPLEGAR	SEQ ID NO.: 43
				LELHVDGPPPRPQLR	SEQ ID NO.: 185
				CEGPIPDVTFELLR	SEQ ID NO.: 38
				NGVAQEPVHLDSPAIK	SEQ ID NO.: 227
				ATWSGAVLAGR	SEQ ID NO.: 31
				HQFLLTGDTQGR	SEQ ID NO.: 147
MSF-236	MSPI-236.2	5.20	72071	VWVYPPEK	SEQ ID NO.: 351
				GECQAEGVLFFQGDR	SEQ ID NO.: 122

The second group comprises MSPIs that are increased in the CSF of subjects having MS as compared with the CSF of subjects free from MS, where the differential presence is significant. The amino acid sequences of tryptic digest peptides of these MSPIs identified by tandem mass spectrometry and database searching are listed in Table V in addition to the pIs and MWs of these MSPIs.

Table V. MSPIs Increased in CSF of Subjects Having MS

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
MSF-237	MSPI-237	10.15	11421	ALDFAVGEYNK	SEQ ID NO.: 19
				LVGGPMDASVEEEGVR	SEQ ID NO.: 211
MSF-238	MSPI-238	8.26	11328	ALDFAVGEYNK	SEQ ID NO.: 19
				LVGGPMDASVEEEGVR	SEQ ID NO.: 211
MSF-239	MSPI-239.1	6.65	14399	AQGFTEDTIVFLPQTDK	SEQ ID NO.: 26
				TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-239	MSPI-239.2	6.65	14399	ALDFAVGEYNK	SEQ ID NO.: 19
				LVGGPMDASVEEEGVR	SEQ ID NO.: 211
MSF-240	MSPI-240.1	7.73	27538	GFQALGDAADIR	SEQ ID NO.: 124
MSF-240	MSPI-240.2	7.73	27538	YAASSYLSLTPEQWK	SEQ ID NO.: 364
				AAPSVTLFPPSSEELQANK	SEQ ID NO.: 2
MSF-241	MSPI-241.1	6.10	86456	AEMADQAAAWLTR	SEQ ID NO.: 8
MSF-241	MSPI-241.2	6.10	86456	QIQVSWLR	SEQ ID NO.: 245
				VSVFVPPR	SEQ ID NO.: 345
				YVTSAPMPEPQAPGR	SEQ ID NO.: 377
MSF-242	MSPI-242.1	7.30	25465	AAPSVTLFPPSSEELQANK	SEQ ID NO.: 2
				YAASSYLSLTPEQWK	SEQ ID NO.: 364
MSF-242	MSPI-242.2	7.30	25465	AQGFTEDTIVFLPQTDK	SEQ ID NO.: 26
				TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-243	MSPI-243	6.01	12948	AADDTWEPFASGK	SEQ ID NO.: 1
MSF-244	MSPI-244	5.27	11037	AQGFTEDTIVFLPQTDK	SEQ ID NO.: 26
MSF-245	MSPI-245	6.21	80579	VSVFVPPR	SEQ ID NO.: 345
				YVTSAPMPEPQAPGR	SEQ ID NO.: 377
MSF-246	MSPI-246.1	8.78	15019	AADDTWEPFASGK	SEQ ID NO.: 1

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
				GSPAINVAVHVFR	SEQ ID NO.: 140
MSF-246	MSPI-246.2	8.78	15019	ALDFAVGEYNK	SEQ ID NO.: 19
				LVGGPMDASVEEEGVR	SEQ ID NO.: 211
MSF-248	MSPI-248	7.19	30921	CSVFYGAPSK	SEQ ID NO.: 47
				VEYGFQVK	SEQ ID NO.: 329
				GLQDEDGYR	SEQ ID NO.: 133
				ITQVLHFTK	SEQ ID NO.: 165
MSF-250	MSPI-250.2	4.33	42755	TEDTIFLR	SEQ ID NO.: 292
MSF-251	MSPI-251	7.60	81385	TIYTPGSTVLYR	SEQ ID NO.: 306
				QELSEAEQATR	SEQ ID NO.: 239
MSF-254	MSPI-254.1	4.28	11270	DQDGEILLPR	SEQ ID NO.: 63
MSF-257	MSPI-257.1	4.64	13792	AADDTWEPFASGK	SEQ ID NO.: 1
				GSPAINVAVHVFR	SEQ ID NO.: 140
MSF-257	MSPI-257.2	4.64	13792	SELEEQLTPVAEETR	SEQ ID NO.: 267
MSF-257	MSPI-257.3	4.64	13792	AADDTWEPFASGK	SEQ ID NO.: 1
				GSPAINVAVHVFR	SEQ ID NO.: 140
MSF-260	MSPI-260.1	6.06	78111	AEMADQAAAWLTR	SEQ ID NO.: 8
MSF-260	MSPI-260.2	6.06	78111	QIQVSWLR	SEQ ID NO.: 245
				VSVFVPPR	SEQ ID NO.: 345
				YVTSAPMPEPQAPGR	SEQ ID NO.: 377
MSF-261	MSPI-261	4.86	44065	WEAEPVYVQR	SEQ ID NO.: 354
				IDVHWTR	SEQ ID NO.: 153
				AGEVQEPELR	SEQ ID NO.: 16
				AREDIFMETLK	SEQ ID NO.: 27
MSF-262	MSPI-262.2	4.40	60275	EFEGEEEYLEILGITR	SEQ ID NO.: 74
				VTVNYPPTITESK	SEQ ID NO.: 348
MSF-265	MSPI-265	6.56	20744	SDGSCAWYR	SEQ ID NO.: 266
				EVDSGNDIYGNPIK	SEQ ID NO.: 97
MSF-266	MSPI-266	6.54	13783	LEEQAQQIR	SEQ ID NO.: 183
MSF-267	MSPI-267	5.54	21908	PPYTVVYFPVR	SEQ ID NO.: 237
				FQDGDLTLYQSNTILR	SEQ ID NO.: 112
				EEVVTVETWQEGSLK	SEQ ID NO.: 73
MSF-268	MSPI-268.1	8.85	13625	ALDFAVGEYNK	SEQ ID NO.: 19
				LVGGPMDASVEEEGVR	SEQ ID NO.: 211
MSF-270	MSPI-270	5.74	32454	AADDTWEPFASGK	SEQ ID NO.: 1
				GSPAINVAVHVFR	SEQ ID NO.: 140
MSF-271	MSPI-271	6.43	45269	TIEAEAAHGTVTR	SEQ ID NO.: 304
MSF-273	MSPI-273	4.94	16019	LGPLVEQGR	SEQ ID NO.: 192
				AATVGSLAGQPLQER	SEQ ID NO.: 4
				LEEQAQQIR	SEQ ID NO.: 183
MSF-274	MSPI-274	9.80	23795	GFQALGDAADIR	SEQ ID NO.: 124
MSF-276	MSPI-276	7.32	13812	LYTLVLTDPDAPSR	SEQ ID NO.: 214
				YVWLVYEQDR	SEQ ID NO.: 378
				GNDISSGTVLSDYVGSGPPK	SEQ ID NO.: 136
				NRPTSISWDGLDSGK	SEQ ID NO.: 234
				YVWLVYEQDRPLK	SEQ ID NO.: 379
MSF-277	MSPI-277	6.29	80131	VSVFVPPR	SEQ ID NO.: 345
MSF-279	MSPI-279	6.69	24664	APEAQVSVQPNFQQDK	SEQ ID NO.: 25
				AQGFTEDTIVFLPQTDK	SEQ ID NO.: 26
				TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-280	MSPI-280.1	6.06	33713	AADDTWEPFASGK	SEQ ID NO.: 1

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
				GSPAINVAVHVFR	SEQ ID NO.: 140
MSF-280	MSPI-280.2	6.06	33713	ELDESLQVAER	SEQ ID NO.: 83
				LFDSDPITVTVPVEVSR	SEQ ID NO.: 189
MSF-283	MSPI-283.1	4.78	43626	WEAEPVYVQR	SEQ ID NO.: 354
MSF-283	MSPI-283.2	4.78	43626	CAEENCFIQK	SEQ ID NO.: 36
				ENEGFTVTAEGK	SEQ ID NO.: 87
				VYAYYNLEESCTR	SEQ ID NO.: 353
				SGSDEVQVGQQR	SEQ ID NO.: 270
				GQGTLSVVTMYHAK	SEQ ID NO.: 138
MSF-284	MSPI-284.1	6.43	26066	TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-284	MSPI-284.2	6.43	26066	TVAAPSVFIFPPSDEQLK	SEQ ID NO.: 314
				LLIYDASTR	SEQ ID NO.: 198
				SGTASVVCLLNNFYPR	SEQ ID NO.: 271
				EIVMTQSPATLSVSPGER	SEQ ID NO.: 81
MSF-285	MSPI-285	5.75	27835	SYSCQVTHEGSTVEK	SEQ ID NO.: 286
MSF-289	MSPI-289	6.74	54791	VLSALQAVQGLLVAQGR	SEQ ID NO.: 338
				SLDFTELDVAAEK	SEQ ID NO.: 276
				ALQDQLVLVAAK	SEQ ID NO.: 22
				LQAILGVPWK	SEQ ID NO.: 204
				FMQAVTGWK	SEQ ID NO.: 111
				DPTFIPAPIQAK	SEQ ID NO.: 62
MSF-290	MSPI-290	4.36	20951	TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
				AQGFTEDTIVFLPQTDK	SEQ ID NO.: 26
MSF-291	MSPI-291.1	4.33	105354	LLDSLPSDTR	SEQ ID NO.: 197
MSF-291	MSPI-291.2	4.33	105354	THPHFVIPYR	SEQ ID NO.: 303
				SQVMTHLR	SEQ ID NO.: 283
				VESLEQEAANER	SEQ ID NO.: 328
				AVIQHFQEK	SEQ ID NO.: 32
				VEAMLNDR	SEQ ID NO.: 323
MSF-292	MSPI-292	5.76	13128	AADDTWEPFASGK	SEQ ID NO.: 1
				GSPAINVAVHVFR	SEQ ID NO.: 140
MSF-295	MSPI-295	4.32	36134	IPTTFENGR	SEQ ID NO.: 163
MSF-298	MSPI-298.1	7.51	24762	EIVLTQSPATLSLSPGER	SEQ ID NO.: 80
				FSGSGSGTDFTLTISR	SEQ ID NO.: 115
				VYACEVTHQGLSSPVTK	SEQ ID NO.: 352
MSF-298	MSPI-298.2	7.51	24762	TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
				AQGFTEDTIVFLPQTDK	SEQ ID NO.: 26
MSF-299	MSPI-299	8.12	54806	QELSEAEQATR	SEQ ID NO.: 239
MSF-300	MSPI-300	4.37	40820	VFQEPLFYEAPR	SEQ ID NO.: 330
				MIELHNQEYR	SEQ ID NO.: 215
				LYGMNEEGWR	SEQ ID NO.: 213
				QVMNGFQNR	SEQ ID NO.: 254
MSF-301	MSPI-301	5.58	32266	AADDTWEPFASGK	SEQ ID NO.: 1
				GSPAINVAVHVFR	SEQ ID NO.: 140
				CPLMVKVLDAVR	SEQ ID NO.: 46
MSF-302	MSPI-302	4.28	21301	APEAQVSVQPNFQQDK	SEQ ID NO.: 25
				TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-303	MSPI-303.2	7.84	65034	VWVYPPEK	SEQ ID NO.: 351
				DYFMPCPGR	SEQ ID NO.: 67
				NFPSPVDAAFR	SEQ ID NO.: 225
				RLWWLDLK	SEQ ID NO.: 258

MSF#	MSPI#	pI	MW (Da)	Amino Acid Sequences of Tryptic Digest Peptides	SEQ ID NO:
				GECQAEGVLFFQGDR	SEQ ID NO.: 122
MSF-305	MSPI-305	9.04	21021	APEAQVSVQPNFQQDK	SEQ ID NO.: 25
				TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-306	MSPI-306	6.86	50636	RLWWLDLK	SEQ ID NO.: 258
				DYFMPCPGR	SEQ ID NO.: 67
				YYCFQGNQFLR	SEQ ID NO.: 380
				VWVYPPEK	SEQ ID NO.: 351
				GECQAEGVLFFQGDR	SEQ ID NO.: 122
MSF-307	MSPI-307	7.48	59646	EMSGSPASGIPVK	SEQ ID NO.: 86
				LNMGITDLQGLR	SEQ ID NO.: 199
				GQIVFMNR	SEQ ID NO.: 139
MSF-308	MSPI-308	7.78	55797	TCPKPDDLPFSTVVPLK	SEQ ID NO.: 290
				TFYEPGEEITYSCK	SEQ ID NO.: 298
				ATVVYQGER	SEQ ID NO.: 30
				EHSSLAFWK	SEQ ID NO.: 77
MSF-311	MSPI-311.1	5.41	58040	RVWELSK	SEQ ID NO.: 262
				DDLYVSDAFHK	SEQ ID NO.: 55
MSF-311	MSPI-311.2	5.41	58040	WLQGSQELPR	SEQ ID NO.: 359
MSF-311	MSPI-311.3	5.41	58040	ALQDQLVLVAAK	SEQ ID NO.: 22
				VLSALQAVQGLLVAQGR	SEQ ID NO.: 338
				DPTFIPAPIQAK	SEQ ID NO.: 62
				SLDFTELDVAAEK	SEQ ID NO.: 276
				FMQAVTGWK	SEQ ID NO.: 111
MSF-313	MSPI-313	5.58	24087	TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-314	MSPI-314	4.84	26797	EKPGVYTNVCR	SEQ ID NO.: 82
				LSELIQPLPLER	SEQ ID NO.: 205
				YTNWIQK	SEQ ID NO.: 374
				GLVSWGNIPCGSK	SEQ ID NO.: 135
MSF-316	MSPI-316	5.51	31894	ASSIIDELFQDR	SEQ ID NO.: 28
MSF-318	MSPI-318	4.57	13499	GSPAINVAVHVFR	SEQ ID NO.: 140
MSF-319	MSPI-319	6.72	59646	LNVITVGPR	SEQ ID NO.: 200
				LFAYPDTHR	SEQ ID NO.: 188
				AFYVNVLNEEQR	SEQ ID NO.: 12
				LSQEDPDYGIR	SEQ ID NO.: 207
MSF-320	MSPI-320	8.16	24182	APEAQVSVQPNFQQDK	SEQ ID NO.: 25
				AQGFTEDTIVFLPQTDK	SEQ ID NO.: 26
				TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-322	MSPI-322	5.21	22551	TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-323	MSPI-323.1	5.21	31216	EILSVDCSTNNPSQAK	SEQ ID NO.: 78
MSF-323	MSPI-323.2	5.21	31216	GSPAINVAVHVFR	SEQ ID NO.: 140
				AADDTWEPFASGK	SEQ ID NO.: 1
MSF-324	MSPI-324	7.10	23117	TMLLQPAGSLGSYSYR	SEQ ID NO.: 311
MSF-325	MSPI-325.1	4.05	43334	VLSLAQEQVGGSPEK	SEQ ID NO.: 339
				QGSFQGGFR	SEQ ID NO.: 244
MSF-325	MSPI-325.2	4.05	43334	ALGHLDLSGNR	SEQ ID NO.: 21
				VAAGAFQGL	SEQ ID NO.: 319
				YLFLNGNK	SEQ ID NO.: 370

As will be evident to one of skill in the art, based upon the present description, a given MSPI can be described according to the data provided for that MSPI in Table IV or V. The MSPI is a polypeptide comprising a peptide sequence described for that MSPI (preferably comprising a plurality of, more preferably all of, the peptide sequences described for that MSPI) and has a pI of about the value stated for that MSPI (preferably within 10%, more preferably within 5% still more preferably within 1% of the stated value) and has a MW of about the value stated for that MSPI (preferably within 10%, more preferably within 5%, still more preferably within 1% of the stated value).

In one embodiment, a first sample of body fluid from a subject is analyzed for quantitative detection of one or more of the MSPIs as defined in Table IV, or any combination of them, wherein a decreased abundance of the MSPI or MSPIs (or any combination of them) in the first sample from the subject relative to the second sample from a subject or subjects free from MS (e.g., a control sample or a previously determined reference range) indicates the presence of MS.

In another embodiment of the invention, a first sample of body fluid from a subject is analyzed for quantitative detection of one or more of the MSPIs as defined in Table V, or any combination of them, wherein an increased abundance of the MSPI or MSPIs (or any combination of them) in first sample from the subject relative to the second sample from a subject or subjects free from MS (e.g., a control sample or a previously determined reference range) indicates the presence of MS.

In a further embodiment, a first sample of body fluid from a subject is analyzed for quantitative detection of (a) one or more MSPIs, or any combination of them, whose decreased abundance indicates the presence of MS, i.e., the MSPIs as defined in Table IV; and (b) one or more MSPIs, or any combination of them, whose increased abundance indicates the presence of MS, i.e., the MSPIs as defined in Table V.

In yet a further embodiment, a first sample of body fluid from a subject is analyzed for quantitative detection of one or more MSPIs and one or more previously known biomarkers of MS (e.g., candidate markers such as hypersensitive platelet glutamate receptors (Berk et al. Int Clin Psychopharmacol 1999 14, 199-122)). In accordance with this embodiment, the abundance of each MSPI and known biomarker relative to a control or reference range indicates whether a subject has MS.

Preferably, the abundance of an MSPI is normalized to an Expression Reference Protein Isoform (ERPI). ERPIs can be identified by partial amino acid sequencing of ERFs, which are described above, using the methods and apparatus of the Preferred Technology. The partial amino acid sequences of an ERPI, and the known proteins to which it is homologous is presented in Table VI.

Table VI. Expression Reference Protein Isoforms

ERF#	ERPI#	Amino Acid Sequences of Tryptic Digest Peptides
ERF-1	ERPI-1.1	RVWELSK, FATTFYQHLADSK, VAEGTQVLELPFK, GDDITMVLILPKPEK,

ERF#	ERPI#	Amino Acid Sequences of Tryptic Digest Peptides
		LPGIVAEGR, DDLYVSDAFHK, EVPLNTIIFMGR
ERF-1	ERPI-1.2	YTFELSR, RTHLPEVFLSK
ERF-2	ERPI-2	FSGTWYAMAK, YWGVASFLQK, QEELCLAR, LIVHNGYCDGR
ERF-3	ERPI-3	SVVAPATDGGLNLTSTFLR, TMLLQPAGSLGSYSYR

The MSPIs described herein include isoforms of known proteins where the isoforms were not previously known to be associated with MS. For each MSPI, the present invention additionally provides: (a) antibodies that bind to said MSPI, to said fragments, or both to said MSPI and to said fragments. Preferably the MSPI is in an isolated form, as used herein, an MSPI is "isolated" when it is present in a preparation that is substantially free of contaminating proteins, i.e., a preparation in which less than 10% (preferably less than 5%, more preferably less than 1%) of the total protein present is contaminating protein(s). A contaminating protein is a protein or protein isoform having a significantly different pI or MW from those of the isolated MSPI, as determined by 2D electrophoresis. As used herein, a "significantly different" pI or MW is one that permits the contaminating protein to be resolved from the MSPI on 2D electrophoresis, performed according to the Reference Protocol.

In one embodiment, an isolated polypeptide is provided, said polypeptide comprising a peptide with the amino acid sequence identified in Table IV or V for an MSPI, said polypeptide having a pI and MW within 10% (preferably within 5%, more preferably within 1%) of the values identified in Table IV or V for that MSPI.

The MSPIs of the invention can be qualitatively or quantitatively detected by any method known to those skilled in the art, including but not limited to the Preferred Technology described herein, kinase assays, enzyme assays, binding assays and other functional assays, immunoassays, and western blotting. In one embodiment, the MSPIs are separated on a 2-D gel by virtue of their MWs and pIs and visualized by staining the gel. In one embodiment, the MSPIs are stained with a fluorescent dye and imaged with a fluorescence scanner. Sypro Red (Molecular Probes, Inc., Eugene, Oregon) is a suitable dye for this purpose. A preferred fluorescent dye is Pyridinium, 4-[2-[4-(dipentylamino)-2-trifluoromethylphenyl] ethenyl]-1-(sulfobutyl)-, inner salt. See U.S. Application No. 09/412,168, filed on October 5, 1999, which is incorporated herein by reference in its entirety.

Alternatively, MSPIs can be detected in an immunoassay. In one embodiment, an immunoassay is performed by contacting a first sample from a subject to be tested with a capture reagent (e.g an antibody) under conditions such that immunospecific binding can occur

if the MSPI is present, and detecting or measuring the amount of any immunospecific binding by the capture reagent. Anti-MSPI antibodies can be produced by the methods and techniques taught herein; examples of such antibodies known in the art are set forth in Table VII. These antibodies shown in Table VII are already known to bind to the protein of which the MSPI is itself a family member. Preferably, the anti-MSPI antibody preferentially binds to the MSPI rather than to other isoforms of the same protein. In a preferred embodiment, the anti-MSPI antibody binds to the MSPI with at least 2-fold greater affinity, more preferably at least 5-fold greater affinity, still more preferably at least 10-fold greater affinity, than to said other isoforms of the same protein.

MSPIs can be transferred from a gel to a suitable membrane (e.g. a PVDF membrane) and subsequently probed in suitable assays that include, without limitation, competitive and non-competitive assay systems using techniques such as western blots and "sandwich" immunoassays using anti-MSPI antibodies as described herein, e.g., the antibodies raised against the MSPIs of interest. The immunoblots can be used to identify those anti-MSPI antibodies displaying the selectivity required to immuno-specifically differentiate an MSPI from other isoforms encoded by the same gene.

Table VII. Known Antibodies That Recognize MSPIs or MSPI-Related Polypeptides or Protein family of which MSPI is a member

MSPI#	Antibody	Manufacturer	Catalogue Number
MSPI-1	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-2	Goat anti-Clusterin (human)	RDI RESEARCH DIAGNOSTICS, INC	RDI-CLUSTRCabG
MSPI-3	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
MSPI-4	C7 Complement, Goat anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BMD- G34
MSPI-5.1	C7 Complement, Goat anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BMD- G34
MSPI-6	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-7	AT1 (306)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-579
MSPI-8.1	Goat anti-Clusterin (human)	RDI RESEARCH DIAGNOSTICS,	RDI-CLUSTRCabG

MSPI#	Antibody	Manufacturer	Catalogue Number
		INC	
MSPI-8.2	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
MSPI-9	rabbit anti-human alpha-2-macroglobulin	Cambio Ltd.	CA-0427
MSPI-10	rabbit anti-human alpha-2-macroglobulin	Cambio Ltd.	CA-0427
MSPI-11	rabbit anti-human alpha-2-macroglobulin	Cambio Ltd.	CA-0427
MSPI-12.1	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-12.2	goat anti-Pyruvate Kinase (polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-7140
MSPI-13	rabbit anti-human alpha-2-macroglobulin	Cambio Ltd.	CA-0427
MSPI-14	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
MSPI-15	rabbit anti-human alpha-2-macroglobulin	Cambio Ltd.	CA-0427
MSPI-16	rabbit anti-human alpha-2-macroglobulin	Cambio Ltd.	CA-0427
MSPI-18	rabbit anti-human alpha-2-macroglobulin	Cambio Ltd.	CA-0427
MSPI-22	rabbit anti-human alpha-2-macroglobulin	Cambio Ltd.	CA-0427
MSPI-24	rabbit anti-human alpha-2-macroglobulin	Cambio Ltd.	CA-0427
MSPI-27	C6 Complement, Goat anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BMD- G33
MSPI-28	rabbit anti-human alpha-2-macroglobulin	Cambio Ltd.	CA-0427
MSPI-29	rabbit anti-human alpha-2-macroglobulin	Cambio Ltd.	CA-0427
MSPI-30	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
MSPI-31	rabbit anti-human alpha-2-macroglobulin	Cambio Ltd.	CA-0427
MSPI-32	C6 Complement, Goat anti-Human	ACCURATE	BMD- G33

MSPI#	Antibody	Manufacturer	Catalogue Number
		CHEMICAL & SCIENTIFIC CORPORATION	
MSPI-33.1	goat anti-Pyruvate Kinase (polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-7140
MSPI-36	goat anti-Pyruvate Kinase (polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-7140
MSPI-37	Rabbit Polyclonal Anti-Human Ceruloplasmin	DAKO CORPORATION	A0031
MSPI-39.1	Goat anti-human Fibulin-1(polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-8674
MSPI-40	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
MSPI-42.2	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
MSPI-47	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
MSPI-48.1	Goat anti-human Fibulin-1(polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-8674
MSPI-51.1	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
MSPI-52.1	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
MSPI-52.2	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL &	IMS- 01-001-02

MSPI#	Antibody	Manufacturer	Catalogue Number
		SCIENTIFIC CORPORATION	
MSPI-54	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-55	Anti-Human Contactin, Mouse monoclonal	BD Biosciences	610579
MSPI-56	Rabbit Polyclonal Anti-Human Ceruloplasmin	DAKO CORPORATION	A0031
MSPI-58	rabbit anti-human alpha-2-macroglobulin	Cambio Ltd.	CA-0427
MSPI-60	Anti-Human Contactin, Mouse monoclonal	BD Biosciences	610579
MSPI-65	C6 Complement, Goat anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BMD- G33
MSPI-67.1	goat anti-Pyruvate Kinase (polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-7140
MSPI-67.2	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-70	C1r Complement, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YSRT- AHC002
MSPI-72	Rabbit Polyclonal Anti-Human Ceruloplasmin	DAKO CORPORATION	A0031
MSPI-75	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
MSPI-76	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
MSPI-78.1	Goat anti-Clusterin (human)	RDI RESEARCH DIAGNOSTICS, INC	RDI-CLUSTRCabG
MSPI-78.2	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-79	Anti-Human Contactin, Mouse monoclonal	BD Biosciences	610579
MSPI-80.1	Monoclonal mouse anti-human IgA1	RDI RESEARCH DIAGNOSTICS, INC	RDI-TRK1A2-2B5
MSPI-81	Transthyretin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL &	MED- CLA 193

MSPI#	Antibody	Manufacturer	Catalogue Number
		SCIENTIFIC CORPORATION	
MSPI-84.1	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
MSPI-89.1	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
MSPI-90	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
MSPI-91.1	Apolipoprotein A1 (HDL), Sheep anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	ACL- 20075AP
MSPI-92	Anti-Human Contactin, Mouse monoclonal	BD Biosciences	610579
MSPI-93.1	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
MSPI-93.2	Goat anti-Clusterin (human)	RDI RESEARCH DIAGNOSTICS, INC	RDI-CLUSTRCabG
MSPI-95	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
MSPI-96	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
MSPI-98	Monoclonal anti-Neuron Specific Enolase	BIODESIGN INTERNATIONAL	M37403M
MSPI-102.1	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
MSPI-102.2	Goat anti-Clusterin (human)	RDI RESEARCH DIAGNOSTICS, INC	RDI-CLUSTRCabG
MSPI-102.3	Lactic Dehydrogenase (LDH) (H-subunit), Clone: HH-17, Mab anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BYA- 6019-1
MSPI-104	Complement Factor B, C3 proactivator, Rabbit anti-Human	ACCURATE CHEMICAL &	AXL- 466/2

MSPI#	Antibody	Manufacturer	Catalogue Number
		SCIENTIFIC CORPORATION	
MSPI-105.1	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-105.2	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
MSPI-106	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
MSPI-107	goat anti-Pyruvate Kinase (polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-7140
MSPI-108	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-110	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
MSPI-111	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-113	Complement Factor B, C3 proactivator, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 466/2
MSPI-114	Rabbit Polyclonal Anti-Human Ceruloplasmin	DAKO CORPORATION	A0031
MSPI-119	goat anti-Pyruvate Kinase (polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-7140
MSPI-120	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
MSPI-122	Rabbit anti-human Insulin Growth Factor Binding Protein 2	RDI RESEARCH DIAGNOSTICS, INC	RDI-IGFBP2abr
MSPI-123.1	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210

MSPI#	Antibody	Manufacturer	Catalogue Number
MSPI-127.1	AT1 (306)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-579
MSPI-128	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
MSPI-130	Goat anti-human Fibulin-1(polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-8674
MSPI-132	Factor H (Complement), Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-066-02
MSPI-133	Transthyretin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA 193
MSPI-134	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
MSPI-137	AT1 (306)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-579
MSPI-140	Tetranectin, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 494
MSPI-141.2	goat anti-Pyruvate Kinase (polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-7140
MSPI-142	AT1 (306)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-579
MSPI-145.2	Rabbit Polyclonal Anti-Human	DAKO	A0031

MSPI#	Antibody	Manufacturer	Catalogue Number
	Ceruloplasmin	CORPORATION	
MSPI-147.2	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
MSPI-149	Complement Factor B, C3 proactivator, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 466/2
MSPI-150	Anti-Human Contactin, Mouse monoclonal	BD Biosciences	610579
MSPI-151	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
MSPI-152	Monoclonal mouse anti-human IgA1	RDI RESEARCH DIAGNOSTICS, INC	RDI-TRK1A2-2B5
MSPI-154.1	Rabbit Polyclonal Anti-Human Ceruloplasmin	DAKO CORPORATION	A0031
MSPI-155.2	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-155.3	goat anti-Pyruvate Kinase (polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-7140
MSPI-156.2	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-158.2	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
MSPI-159	Complement Factor B, C3 proactivator, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 466/2
MSPI-160	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-161	AT1 (306)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-579
MSPI-165.2	goat anti-Pyruvate Kinase (polyclonal)	SANTA CRUZ BIOTECHNOLOG	sc-7140

MSPI#	Antibody	Manufacturer	Catalogue Number
		Y, INC - RESEARCH ANTIBODIES 98/99	
MSPI-167	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
MSPI-169.2	Rabbit Polyclonal Anti-Human Ceruloplasmin	DAKO CORPORATION	A0031
MSPI-170	Rabbit Polyclonal Anti-Human Ceruloplasmin	DAKO CORPORATION	A0031
MSPI-171	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
MSPI-172	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
MSPI-173.2	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
MSPI-175	Goat anti-human Fibulin-1(polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-8674
MSPI-176.1	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-176.2	goat anti-Pyruvate Kinase (polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-7140
MSPI-177	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
MSPI-178	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-179.1	Monoclonal mouse anti-human IgA1	RDI RESEARCH DIAGNOSTICS, INC	RDI-TRK1A2-2B5
MSPI-179.2	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC	YN- RHHPX

MSPI#	Antibody	Manufacturer	Catalogue Number
		CORPORATION	
MSPI-181.2	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-182	AT1 (306)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-579
MSPI-183	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-184	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-186	goat anti-Pyruvate Kinase (polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-7140
MSPI-187.1	Goat anti-Clusterin (human)	RDI RESEARCH DIAGNOSTICS, INC	RDI-CLUSTRCabG
MSPI-187.2	Transthyretin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA 193
MSPI-189	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
MSPI-191	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
MSPI-192.1	Tetranectin, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 494
MSPI-193	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-194.1	Monoclonal mouse anti-human plasminogen	RDI RESEARCH DIAGNOSTICS, INC	RDI-TRK4P11-4D2
MSPI-195.1	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
MSPI-196	AT1 (306)	SANTA CRUZ BIOTECHNOLOGY, INC -	sc-579

MSPI#	Antibody	Manufacturer	Catalogue Number
		RESEARCH ANTIBODIES 98/99	
MSPI-197	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-198	Tetranectin, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 494
MSPI-199.1	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-199.2	goat anti-Pyruvate Kinase (polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-7140
MSPI-200	Monoclonal mouse anti-human plasminogen	RDI RESEARCH DIAGNOSTICS, INC	RDI-TRK4P11-4D2
MSPI-201	Transthyretin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA 193
MSPI-202.1	Prothrombin, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 448/2
MSPI-205	Transthyretin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA 193
MSPI-206	Apolipoprotein D, Clone: 36C6, Mab anti-Human, paraffin, IH/WB	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA457
MSPI-209	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-210	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-212.2	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
MSPI-213	Monoclonal anti-human Fibrinogen	BIODESIGN INTERNATIONAL	N77190M
MSPI-215	Goat anti-Clusterin (human)	RDI RESEARCH	RDI-

MSPI#	Antibody	Manufacturer	Catalogue Number
		DIAGNOSTICS, INC	CLUSTRCabG
MSPI-216	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
MSPI-217.3	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
MSPI-218	Goat anti-human Fibulin-1(polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-8674
MSPI-219	Monoclonal mouse anti-human plasminogen	RDI RESEARCH DIAGNOSTICS, INC	RDI-TRK4P11-4D2
MSPI-220	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-221.1	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
MSPI-222.2	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-223.1	Mouse anti-Human pigment epithelium-derived factor (PEDF) (monoclonal) Clone 10F12.2	Chemicon International	MAB1059
MSPI-224.1	Mouse anti-human complement component C9 (monoclonal)	RDI RESEARCH DIAGNOSTICS, INC	RDI-COMPC9abm-A6
MSPI-228.1	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
MSPI-228.2	Alpha-1-Acid Glycoprotein, Clone: AGP-47, Mab anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BYA- 6189-1
MSPI-229.2	Chicken polyclonal to human mu-chain	Abcam Ltd.	ab453-1000
MSPI-230	Gelsolin, plasma + cytoplasmic, Sheep anti-	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YBG- 4628-6210
MSPI-231	goat anti-Pyruvate Kinase (polyclonal)	SANTA CRUZ BIOTECHNOLOGY, INC -	sc-7140

MSPI#	Antibody	Manufacturer	Catalogue Number
		RESEARCH ANTIBODIES 98/99	
MSPI-232	Apolipoprotein D, Clone: 36C6, Mab anti-Human, paraffin, IH/WB	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA457
MSPI-233.1	Goat anti-Clusterin (human)	RDI RESEARCH DIAGNOSTICS, INC	RDI-CLUSTRCabG
MSPI-233.2	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
MSPI-234	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-235	Tissue Inhibitor of Matrix Metalloproteinase 2 (TIMP2) (NO X w/TIMP1), Clone: 3A4, Mab anti-Human, paraffin, IH	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA498
MSPI-236.1	Alpha-1-Acid Glycoprotein, Clone: AGP-47, Mab anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BYA- 6189-1
MSPI-236.2	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
MSPI-237	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
MSPI-238	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574.
MSPI-239.1	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-239.2	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
MSPI-240.1	Tissue Inhibitor of Matrix Metalloproteinase 1 (TIMP1) (NO X w/TIMP2) Clone: 2A5, Mab anti-Human, paraffin, IH	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA497
MSPI-240.2	Monoclonal to human lambda light chain	Diatec.com	1020
MSPI-241.2	Chicken polyclonal to human mu-chain	Abcam Ltd.	ab453-1000
MSPI-242.2	Anti-prostaglandin D	Oxford Biomedical	PD-01

MSPI#	Antibody	Manufacturer	Catalogue Number
	synthase/Prostaglandin H2-D isomerase	Research	
MSPI-243	Transthyretin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA 193
MSPI-244	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-245	Chicken polyclonal to human mu-chain	Abcam Ltd.	ab453-1000
MSPI-246.1	Transthyretin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA 193
MSPI-246.2	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
MSPI-248	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
MSPI-251	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
MSPI-257.2	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
MSPI-257.3	Transthyretin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA 193
MSPI-260.2	Chicken polyclonal to human mu-chain	Abcam Ltd.	ab453-1000
MSPI-265	Tissue Inhibitor of Matrix Metalloproteinase 2 (TIMP2) (NO X w/TIMP1), Clone: 3A4, Mab anti-Human, paraffin, IH	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA498
MSPI-266	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human, frozen/paraffin	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YM- 5029
MSPI-268.1	Cystatin C, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	AXL- 574
MSPI-270	Transthyretin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA 193
MSPI-273	Apolipoprotein E, LDL, VLDL, Clone: 3D12, Mab anti-Human,	ACCURATE CHEMICAL &	YM- 5029

MSPI#	Antibody	Manufacturer	Catalogue Number
	frozen/paraffin	SCIENTIFIC CORPORATION	
MSPI-274	Tissue Inhibitor of Matrix Metalloproteinase 1 (TIMP1) (NO X w/TIMP2) Clone: 2A5, Mab anti-Human, paraffin, IH	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA497
MSPI-277	Chicken polyclonal to human mu-chain	Abcam Ltd.	ab453-1000
MSPI-279	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-280.2	Goat anti-Clusterin (human)	RDI RESEARCH DIAGNOSTICS, INC	RDI-CLUSTRCabG
MSPI-283.2	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
MSPI-284.1	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-289	AT1 (306)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-579
MSPI-290	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-291.2	Anti-Alzheimer precursor protein A4	RDI RESEARCH DIAGNOSTICS, INC	RDI-ALZHPA4abm
MSPI-292	Transthyretin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA 193
MSPI-295	Apolipoprotein D, Clone: 36C6, Mab anti-Human, paraffin, IH/WB	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA457
MSPI-298.2	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-299	C3 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-001-02
MSPI-301	Transthyretin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA 193
MSPI-302	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-303.2	Hemopexin, Beta-1, Rabbit anti-	ACCURATE	YN- RHHPX

MSPI#	Antibody	Manufacturer	Catalogue Number
	Human, precipitating	CHEMICAL & SCIENTIFIC CORPORATION	
MSPI-305	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-306	Hemopexin, Beta-1, Rabbit anti-Human, precipitating	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	YN- RHHPX
MSPI-307	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	IMS- 01-032-02
MSPI-311.1	Antithrombin III, Clone: BL-ATIII/3, Mab anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	BYA- 9009-1
MSPI-311.2	Monoclonal mouse anti-human IgA1	RDI RESEARCH DIAGNOSTICS, INC	RDI-TRK1A2-2B5
MSPI-311.3	AT1 (306)	SANTA CRUZ BIOTECHNOLOGY, INC - RESEARCH ANTIBODIES 98/99	sc-579
MSPI-313	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-316	Goat anti-Clusterin (human)	RDI RESEARCH DIAGNOSTICS, INC	RDI-CLUSTRCabG
MSPI-318	Transthyretin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA 193
MSPI-319	Rabbit anti-Catalase (polyclonal)	Abcam Ltd.	ab6572
MSPI-320	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-322	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-323.1	Goat anti-Clusterin (human)	RDI RESEARCH DIAGNOSTICS, INC	RDI-CLUSTRCabG
MSPI-323.2	Transthyretin, Prealbumin, 55kD, Rabbit anti-Human	ACCURATE CHEMICAL & SCIENTIFIC CORPORATION	MED- CLA 193
MSPI-324	Anti-prostaglandin D synthase/Prostaglandin H2-D isomerase	Oxford Biomedical Research	PD-01
MSPI-325.1	C4 Complement, Chicken anti-Human	ACCURATE CHEMICAL &	IMS- 01-032-02

MSPI#	Antibody	Manufacturer	Catalogue Number
		SCIENTIFIC CORPORATION	

*Further information about these antibodies can be obtained from their commercial sources at: ACCURATE CHEMICAL & SCIENTIFIC CORPORATION http://www.accuratechemical.com/; BIODESIGN INTERNATIONAL - http://www.biodesign.com/; RDI RESEARCH DIAGNOSTICS, INC - http://www.researchd.com/;SANTA CRUZ BIOTECHNOLOGY, INC - http://www.scbt.com/.

As used herein, an "aberrant level" means a level that is increased or decreased in a first sample compared with the level in a second sample from a subject free from MS or a reference level. In one embodiment, binding of antibody in tissue sections can be used to detect aberrant MSPI localization or an aberrant level of one or more MSPIs. In a specific embodiment, antibody to an MSPI can be used to assay a first tissue sample (e.g., a brain biopsy) from a subject for the level of the MSPI where an aberrant level of MSPI is indicative of MS.

Any suitable immunoassay can be used, including, without limitation, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays and protein A immunoassays.

For example, an MSPI can be detected in a fluid sample (e.g., CSF, blood, urine, or tissue homogenate) by means of a two-step sandwich assay. In the first step, a capture reagent (e.g., an anti-MSPI antibody) is used to capture the MSPI. Examples of such antibodies known in the art are set forth in Table VII. The capture reagent can optionally be immobilized on a solid phase. In the second step, a directly or indirectly labeled detection reagent is used to detect the captured MSPI. In one embodiment, the detection reagent is a lectin. Any lectin can be used for this purpose that preferentially binds to the MSPI rather than to other isoforms that have the same core protein as the MSPI or to other polypeptides that share the antigenic determinant recognized by the antibody. In a preferred embodiment, the chosen lectin binds to the MSPI with at least 2-fold greater affinity, more preferably at least 5-fold greater affinity, still more preferably at least 10-fold greater affinity, than to said other isoforms that have the same core protein as the MSPI or to said other polypeptides that share the antigenic determinant recognized by the antibody. Based on the present description, a lectin that is suitable for detecting a given MSPI can readily be identified by methods well known in the art, for instance upon testing one or more lectins enumerated in Table I on pages 158-159 of Sumar et al., Lectins as Indicators of Disease-Associated Glycoforms, In: Gabius H-J & Gabius S (eds.), 1993, Lectins and Glycobiology, at pp. 158-174 (which is incorporated herein by reference in its entirety). Lectins with the desired oligosaccharide specificity can be identified, for example, by their ability to detect the MSPI in a 2D gel, in a replica of a 2D gel following transfer to a suitable solid substrate such as a nitrocellulose membrane, or in a two-step assay following capture by capture reagent. In an alternative embodiment, the detection reagent is an antibody, e.g., an antibody that immunospecifically detects

post-translational modifications, such as an antibody that immunospecifically binds to phosphorylated amino acids. Examples of such antibodies include those that bind to phosphotyrosine (BD Transduction Laboratories, 2002, catalog nos.:P11120; P39020), those that bind to phosphoserine (Zymed Laboratories Inc. 2002, South San Francisco, CA, catalog no. 61-8100) and those that bind to phosphothreonine (Zymed Laboratories Inc., 2002, South San Francisco, CA, catalog nos. 71-8200, 13-9200).

If desired, a gene encoding an MSPI, a related gene, or related nucleic acid sequences or subsequences, including complementary sequences, can also be used in hybridization assays. A nucleotide encoding an MSPI, or subsequences thereof comprising at least 8 nucleotides, preferably at least 12 nucleotides, and most preferably at least 15 nucleotides can be used as a hybridization probe. Hybridization assays can be used for detection, prognosis, diagnosis, or monitoring of conditions, disorders, or disease states, associated with aberrant expression of genes encoding MSPIs, or for differential diagnosis of subjects with signs or symptoms suggestive of MS. In particular, such a hybridization assay can be carried out by a method comprising contacting a subject's sample containing nucleic acid with a nucleic acid probe capable of hybridizing to a DNA or RNA that encodes an MSPI, under conditions such that hybridization can occur, and detecting or measuring any resulting hybridization. Nucleotides can be used for therapy of subjects having MS, as described below.

The invention also provides diagnostic kits, comprising an anti-MSPI antibody. In addition, such a kit may optionally comprise one or more of the following: (1) instructions for using the anti-MSPI antibody for diagnosis, prognosis, therapeutic monitoring or any combination of these applications; (2) a labeled binding partner to the antibody; (3) a solid phase (such as a reagent strip) upon which the anti-MSPI antibody is immobilized; and (4) a label or insert indicating regulatory approval for diagnostic, prognostic or therapeutic use or any combination thereof. If no labeled binding partner to the antibody is provided, the anti-MSPI antibody itself can be labeled with a detectable marker, e.g., a chemiluminescent, enzymatic, fluorescent, or radioactive moiety.

The invention also provides a kit comprising a nucleic acid probe capable of hybridizing to RNA encoding an MSPI. In a specific embodiment, a kit comprises in one or more containers a pair of primers (e.g., each in the size range of 6-30 nucleotides, more preferably 10-30 nucleotides and still more preferably 10-20 nucleotides) that under appropriate reaction conditions can prime amplification of at least a portion of a nucleic acid encoding an MSPI, such as by polymerase chain reaction (see, e.g., Innis et al., 1990, PCR Protocols, Academic Press, Inc., San Diego, CA), ligase chain reaction (see EP 320,308) use of Q replicase, cyclic probe reaction, or other methods known in the art.

Kits are also provided which allow for the detection of a plurality of MSPIs or a plurality of nucleic acids each encoding an MSPI. A kit can optionally further comprise a predetermined amount of an isolated MSPI protein or a nucleic acid encoding an MSPI, e.g., for use as a standard or control.

5.5 STATISTICAL TECHNIQUES FOR IDENTIFYING MSFs AND MSF CLUSTERS

The uni-variate differential analysis tools, such as fold changes, wilcoxon rank sum test and t-test, are useful in identifying individual MSFs or MSPIs that are diagnostically

associated with MS or in identifying individual MSPIs that regulate the disease process. In most cases, however, those skilled in the art appreciate that the disease process is associated with a combination of MSFs or MSPIs (and to be regulated by a combination of MSPIs), rather than individual MSFs and MSPIs in isolation. The strategies for discovering such combinations of MSFs and MSPIs differ from those for discovering individual MSFs and MSPIs. In such cases, each individual MSF and MSPIs can be regarded as one variable and the disease can be regarded as a joint, multi-variate effect caused by interaction of these variables.

The following steps can be used to identify markers from data produced by the Preferred Technology.

The first step is to identify a collection of MSFs or MSPIs that individually show a significant aberrant expression in MS. The association between the identified MSFs or MSPIs and MS need not be as highly significant as is desirable when an individual MSF or MSPI is used as a diagnostic. Any of the tests discussed above (fold changes, Wilcoxon rank sum test, etc.) can be used at this stage. Once a suitable collection of MSFs or MSPIs has been identified, a sophisticated multi-variate analysis capable of identifying clusters can then be used to estimate the significant multivariate associations with MS.

Linear Discriminant Analysis (LDA) is one such procedure, which can be used to detect significant association between a cluster of variables (i.e., MSFs or MSPIs) and MS. In performing LDA, a set of weights is associated with each variable (i.e., MSF or MSPI) so that the linear combination of weights and the measured values of the variables can identify the disease state by discriminating between subjects having MS and subjects free from MS. Enhancements to the LDA allow stepwise inclusion (or removal) of variables to optimize the discriminant power of the model. The result of the LDA is therefore a cluster of MSFs or MSPIs which can be used, without limitation, for diagnosis, prognosis, therapy or drug development. Other enhanced variations of LDA, such as Flexible Discriminant Analysis permit the use of non-linear combinations of variables to discriminate a disease state from a normal state. The results of the discriminant analysis can be verified by post-hoc tests and also by repeating the analysis using alternative techniques such as classification trees.

A further category of MSFs or MSPIs can be identified by qualitative measures by comparing the percentage feature presence of an MSF or MSPI of a first sample or sample set (e.g., samples from diseased subjects) with the percentage feature presence of an MSF or MSPI in a second sample or sample set (e.g., samples from control subjects). The "percentage feature presence" of an MSF or MSPI is the percentage of samples in a sample set in which the MSF or MSPI is detectable by the detection method of choice. For example, if an MSF is detectable in 95 percent of samples from diseased subjects, the percentage feature presence of that MSF in that sample set is 95 percent. If only 5 percent of samples from non-diseased subjects have detectable levels of the same MSF, detection of that MSF in the sample of a subject would suggest that it is likely that the subject suffers from MS.

5.6 USE IN CLINICAL STUDIES

The diagnostic methods and compositions of the present invention can assist in monitoring a clinical study, e.g. to evaluate drugs for therapy of MS. In one embodiment, candidate molecules are tested for their ability to restore MSF or MSPI levels in a

subjecthaving MS to levels found in subjects free from MS or, in a treated subject (e.g. after treatment for relapsing-remitting MS with include Interferon β-1b (Betaseron™, Betaferon™), Interferon β-1a (Avonex™, Rebif™), Glatiramer acetate (Copaxone™), intravenous immunoglobulin and, for incidences of acute relapse, therapies with corticosteroids (Noseworthy (1999) Nature 399:suppl. A40-A47), to preserve MSF or MSPI levels at or near non-MS values. The levels of one or more MSFs or MSPIs can be assayed.

In another embodiment, the methods and compositions of the present invention are used to screen candidates for a clinical study to identify individuals having MS; such individuals can then be either excluded from or included in the study or can be placed in a separate cohort for treatment or analysis. If desired, the candidates can concurrently be screened to identify individuals with MS; procedures for these screens are well known in the art.

5.7 PURIFICATION OF MSPIs

In particular aspects, the invention provides isolated mammalian MSPI, preferably human MSPI, and fragments thereof which comprise an antigenic determinant (i.e., can be recognized by an antibody) or which are otherwise functionally active, as well as nucleic acid sequences encoding the foregoing. "Functionally active" as used herein refers to material displaying one or more functional activities associated with a full-length (wild-type) MSPI, e.g., binding to an MSPI substrate or MSPI binding partner, antigenicity (binding to an anti-MSPI antibody), immunogenicity, enzymatic activity and the like.

In specific embodiments, the invention provides fragments of an MSPI comprising at least 5 amino acids, at least 10 amino acids, at least 50 amino acids, or at least 75 amino acids. Fragments lacking some or all of the regions of an MSPI are also provided, as are polypeptides (e.g., fusion proteins) comprising such fragments. Nucleic acids encoding the foregoing are provided.

Once a recombinant nucleic acid which encodes the MSPI, MSPI fragment, or a precursor of the MSPI is identified, the gene product can be analyzed. This is achieved by assays based on the physical or functional properties of the product, including radioactive labeling of the product followed by analysis by gel electrophoresis, immunoassay, etc.

The MSPIs identified herein can be isolated and purified by standard methods including chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Alternatively, once a recombinant nucleic acid that encodes the MSPI is identified, the entire amino acid sequence of the MSPI can be deduced from the nucleotide sequence of the gene coding region contained in the recombinant nucleic acid. As a result, the protein can be synthesized by standard chemical methods known in the art (e.g., see Hunkapiller et al., 1984, Nature 310:105-111).

In another alternative embodiment, native MSPIs can be purified from natural sources, by standard methods such as those described above (e.g., immunoaffinity purification).

In a preferred embodiment, MSPIs are isolated by the Preferred Technology described supra. For preparative-scale runs, a narrow-range "zoom gel" having a pH range of 2 pH units or less is preferred for the isoelectric step, according to the method described in Westermeier,

1993, Electrophoresis in Practice (VCH, Weinheim, Germany), pp. 197-209 (which is incorporated herein by reference in its entirety); this modification permits a larger quantity of a target protein to be loaded onto the gel, and thereby increases the quantity of isolated MSPIs that can be recovered from the gel. When used in this way for preparative-scale runs, the Preferred Technology typically provides up to 100 ng, and can provide up to 1000 ng, of an isolated MSPI in a single run. Those of skill in the art will appreciate that a zoom gel can be used in any separation strategy which employs gel isoelectric focusing.

The invention thus provides an MSPI, MSPI fragment, MSPI-related polypeptide or the MSPI-fusion protein; any of the foregoing can be produced by recombinant DNA techniques or by chemical synthetic methods.

5.8 ISOLATION OF DNA ENCODING AN MSPI

Specific embodiments for the cloning of a gene encoding an MSPI are presented below by way of example and not of limitation.

The nucleotide sequences of the present invention, including DNA and RNA, and comprising a sequence encoding an MSPI, MSPI fragment, MSPI-related polypeptide or the MSPI-fusion protein, may be synthesized using methods known in the art, such as using conventional chemical approaches or polymerase chain reaction (PCR) amplification. The nucleotide sequences of the present invention also permit the identification and cloning of the gene encoding an MSPI homolog or MSPI ortholog including, for example, by screening cDNA libraries, genomic libraries or expression libraries.

For example, to clone a gene encoding an MSPI by PCR techniques, anchored degenerate oligonucleotides (or a set of most likely oligonucleotides) can be designed for all MSPI peptide fragments identified as part of the same protein. PCR reactions under a variety of conditions can be performed with relevant cDNA and genomic DNAs (e.g., from CSF or from cells of the immune system) from one or more species. Also vectorette reactions can be performed on any available cDNA and genomic DNA using the oligonucleotides (which preferably are nested) as above. Vectorette PCR is a method that enables the amplification of specific DNA fragments in situations where the sequence of only one primer is known. Thus, it extends the application of PCR to stretches of DNA where the sequence information is only available at one end. (Arnold C, 1991, PCR Methods Appl. 1(1):39-42; Dyer KD, Biotechniques, 1995, 19(4):550-2). Vectorette PCR may be performed with probes that are, for example, anchored degenerate oligonucleotides (or most likely oligonucleotides) coding for MSPI peptide fragments, using as a template a genomic library or cDNA library pools.

Anchored degenerate oligonucleotides (and most likely oligonucleotides) can be designed for all MSPI peptide fragments. These oligonucleotides may be labeled and hybridized to filters containing cDNA and genomic DNA libraries. Oligonucleotides to different peptides from the same protein will often identify the same members of the library. The cDNA and genomic DNA libraries may be obtained from any suitable or desired mammalian species, for example from humans.

Nucleotide sequences comprising a nucleotide sequence encoding MSPI, MSPI fragment, MSPI-related polypeptide or the MSPI-fusion protein of the present invention are useful for their ability to hybridize selectively with complementary stretches of genes encoding other proteins. Depending on the application, a variety of hybridization conditions may be

employed to obtain nucleotide sequences at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical, or 100% identical, to the sequence of a nucleotide encoding an MSPI.

For a high degree of selectivity, relatively stringent conditions are used to form the duplexes, such as low salt or high temperature conditions. Hybridization conditions can also be rendered more stringent by the addition of increasing amounts of formamide, to destabilize the hybrid duplex. Thus, particular hybridization conditions can be readily manipulated, and will generally be chosen depending on the desired results. In general, convenient hybridization temperatures in the presence of 50% formamide are: 42°C for a probe which is 95 to 100% identical to the fragment of a gene encoding an MSPI, 37°C for 90 to 95% identity and 32°C for 70 to 90% identity.

In the preparation of genomic libraries, DNA fragments are generated, some of which will encode parts or the whole of an MSPI. Any suitable method for preparing DNA fragments may be used in the present invention. For example, the DNA may be cleaved at specific sites using various restriction enzymes. Alternatively, one may use DNAse in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by sonication. The DNA fragments can then be separated according to size by standard techniques, including but not limited to agarose and polyacrylamide gel electrophoresis, column chromatography and sucrose gradient centrifugation. The DNA fragments can then be inserted into suitable vectors, including but not limited to plasmids, cosmids, bacteriophages lambda or T4, and yeast artificial chromosome (YAC). (See, e.g., Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II; Ausubel F.M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York). The genomic library may be screened by nucleic acid hybridization to labeled probe (Benton and Davis, 1977, Science 196:180; Grunstein and Hogness, 1975, Proc. Natl. Acad. Sci. U.S.A. 72:3961).

Based on the present description, the genomic libraries may be screened with labeled degenerate oligonucleotide probes corresponding to the amino acid sequence of any peptide of the MSPI using optimal approaches well known in the art. Any probe used is at least 10 nucleotides, at least 15 nucleotides, at least 20 nucleotides, at least 25 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides, or at least 100 nucleotides.

In Tables IV and V above, some MSPIs disclosed herein were found to correspond to isoforms of previously identified proteins encoded by genes whose sequences are publicly known. (Sequence analysis and protein identification of MSPIs was carried out using the methods described in Section 6.1.14). To screen such a gene, any probe may be used that is complementary to the gene or its complement; preferably the probe is 10 nucleotides or longer, more preferably 15 nucleotides or longer. The SWISS-PROT and trEMBL databases (held by the Swiss Institute of Bioinformatics (SIB) and the European Bioinformatics Institue (EBI) which are available at http://www.expasy.com/) and the GenBank database (held by the National Institute of Health (NIH) which is available at http://www.ncbi.nlm.nih.gov/GenBank/) provide protein sequences for the MSPIs listed in

Tables IV and V under the following accession numbers and each sequence is incorporated herein by reference:

Table VIII. Nucleotide sequences encoding MSPIs, MSPI Related Proteins or ERPIs

MSF#	MSPI#	Accession #
MSF-1	MSPI-1	P41222
MSF-2	MSPI-2	P10909
MSF-3	MSPI-3	P01034
MSF-4	MSPI-4	P10643
MSF-5	MSPI-5.1	P10643
MSF-6	MSPI-6	P41222
MSF-7	MSPI-7	P01019
MSF-8	MSPI-8.1	P10909
MSF-8	MSPI-8.2	P02649
MSF-9	MSPI-9	P01023
MSF-10	MSPI-10	P01023
MSF-11	MSPI-11	P01023
MSF-12	MSPI-12.1	P36955
MSF-12	MSPI-12.2	2745741
MSF-13	MSPI-13	P01023
MSF-14	MSPI-14	P01028
MSF-15	MSPI-15	P01023
MSF-16	MSPI-16	P01023
MSF-18	MSPI-18	P01023
MSF-20	MSPI-20	Q06830
MSF-21	MSPI-21	P25311
MSF-22	MSPI-22	P01023
MSF-24	MSPI-24	P01023
MSF-25	MSPI-25	Q15113
MSF-27	MSPI-27	P13671
MSF-28	MSPI-28	P01023
MSF-29	MSPI-29	P01023
MSF-30	MSPI-30	P01028
MSF-31	MSPI-31	P01023
MSF-32	MSPI-32	P13671
MSF-33	MSPI-33.1	2745741
MSF-33	MSPI-33.2	Q12805
MSF-34	MSPI-34	P32119
MSF-36	MSPI-36	2745741
MSF-37	MSPI-37	P00450
MSF-39	MSPI-39.1	P23142
MSF-39	MSPI-39.2	P14625
MSF-40	MSPI-40	P01034
MSF-42	MSPI-42.2	P02790
MSF-43	MSPI-43	P25311
MSF-44	MSPI-44	P08294
MSF-47	MSPI-47	P02790
MSF-48	MSPI-48.1	P23142
MSF-48	MSPI-48.2	P14625
MSF-48	MSPI-48.3	P14625

MSF#	MSPI#	Accession #
MSF-49	MSPI-49.1	P51693
MSF-49	MSPI-49.2	Q12805
MSF-51	MSPI-51.1	P01034
MSF-52	MSPI-52.1	P01024
MSF-52	MSPI-52.2	P01024
MSF-54	MSPI-54	P41222
MSF-55	MSPI-55	Q12860
MSF-56	MSPI-56	P00450
MSF-58	MSPI-58	P01023
MSF-60	MSPI-60	Q12860
MSF-62	MSPI-62.2	P08603
MSF-63	MSPI-63	2190958
MSF-64	MSPI-64.1	P08603
MSF-65	MSPI-65	P13671
MSF-66	MSPI-66	179674
MSF-67	MSPI-67.1	2745741
MSF-67	MSPI-67.2	P36955
MSF-69	MSPI-69	P13591
MSF-70	MSPI-70	P00736
MSF-72	MSPI-72	P00450
MSF-73	MSPI-73	861521
MSF-75	MSPI-75	P01034
MSF-76	MSPI-76	P01024
MSF-77	MSPI-77.2	P08603
MSF-78	MSPI-78.1	P10909
MSF-78	MSPI-78.2	P41222
MSF-79	MSPI-79	Q12860
MSF-80	MSPI-80.1	P01876
MSF-81	MSPI-81	P02766
MSF-82	MSPI-82	O15394
MSF-83	MSPI-83	Q9Y2T3
MSF-84	MSPI-84.1	P01028
MSF-84	MSPI-84.2	O15394
MSF-86	MSPI-86	Q92876
MSF-87	MSPI-87	Q99435
MSF-89	MSPI-89.1	P02790
MSF-90	MSPI-90	P06396
MSF-91	MSPI-91.1	P02647
MSF-92	MSPI-92	Q12860
MSF-93	MSPI-93.1	P02649
MSF-93	MSPI-93.2	P10909
MSF-94	MSPI-94	P08603
MSF-95	MSPI-95	P01024
MSF-96	MSPI-96	P06396
MSF-97	MSPI-97	P02679
MSF-98	MSPI-98	P06733
MSF-99	MSPI-99	Q99435
MSF-102	MSPI-102.1	P02649
MSF-102	MSPI-102.2	P10909
MSF-102	MSPI-102.3	P07195
MSF-104	MSPI-104	P00751

MSF#	MSPI#	Accession #
MSF-105	MSPI-105.1	P41222
MSF-105	MSPI-105.2	P06396
MSF-106	MSPI-106	P01024
MSF-107	MSPI-107	2745741
MSF-108	MSPI-108	P36955
MSF-110	MSPI-110	P06396
MSF-111	MSPI-111	P36955
MSF-112	MSPI-112	P02749
MSF-113	MSPI-113	P00751
MSF-114	MSPI-114	P00450
MSF-116	MSPI-116	2511666
MSF-117	MSPI-117	7662374
MSF-119	MSPI-119	2745741
MSF-120	MSPI-120	P06396
MSF-122	MSPI-122	P18065
MSF-123	MSPI-123.1	P06396
MSF-125	MSPI-125.1	P08294
MSF-125	MSPI-125.2	7416827
MSF-126	MSPI-126.1	179674
MSF-126	MSPI-126.2	P17900
MSF-127	MSPI-127.1	P01019
MSF-128	MSPI-128	P01024
MSF-130	MSPI-130	P23142
MSF-131	MSPI-131	P05154
MSF-132	MSPI-132	Q03591
MSF-133	MSPI-133	P02766
MSF-134	MSPI-134	P06396
MSF-135	MSPI-135	2190958
MSF-136	MSPI-136	Q15818
MSF-137	MSPI-137	P01019
MSF-139	MSPI-139	P08294
MSF-140	MSPI-140	P05452
MSF-141	MSPI-141.1	P02774
MSF-141	MSPI-141.2	2745741
MSF-142	MSPI-142	P01019
MSF-143	MSPI-143.1	X82321.1
MSF-144	MSPI-144	P02763
MSF-145	MSPI-145.2	P00450
MSF-147	MSPI-147.2	P01024
MSF-148	MSPI-148	Q14624
MSF-149	MSPI-149	P00751
MSF-150	MSPI-150	Q12860
MSF-151	MSPI-151	P01034
MSF-152	MSPI-152	P01876
MSF-154	MSPI-154.1	P00450
MSF-154	MSPI-154.2	2511666
MSF-154	MSPI-154.3	Q99435
MSF-155	MSPI-155.1	P02679
MSF-155	MSPI-155.2	P36955
MSF-155	MSPI-155.3	2745741
MSF-156	MSPI-156.2	P36955

MSF#	MSPI#	Accession #
MSF-158	MSPI-158.2	P02790
MSF-159	MSPI-159	P00751
MSF-160	MSPI-160	P36955
MSF-161	MSPI-161	P01019
MSF-163	MSPI-163	2511666
MSF-165	MSPI-165.1	P02679
MSF-165	MSPI-165.2	2745741
MSF-167	MSPI-167	P02790
MSF-168	MSPI-168	179674
MSF-169	MSPI-169.1	Q14624
MSF-169	MSPI-169.2	P00450
MSF-170	MSPI-170	P00450
MSF-171	MSPI-171	P02649
MSF-172	MSPI-172	P01024
MSF-173	MSPI-173.2	P02790
MSF-175	MSPI-175	P23142
MSF-176	MSPI-176.1	P36955
MSF-176	MSPI-176.2	2745741
MSF-177	MSPI-177	P01024
MSF-178	MSPI-178	P41222
MSF-179	MSPI-179.1	P01876
MSF-179	MSPI-179.2	P02790
MSF-180	MSPI-180	P19021
MSF-181	MSPI-181.1	P17900
MSF-181	MSPI-181.2	P41222
MSF-182	MSPI-182	P01019
MSF-183	MSPI-183	P41222
MSF-184	MSPI-184	P36955
MSF-185	MSPI-185	P01009
MSF-186	MSPI-186	2745741
MSF-187	MSPI-187.1	P10909
MSF-187	MSPI-187.2	P02766
MSF-188	MSPI-188.2	O15394
MSF-189	MSPI-189	P02790
MSF-191	MSPI-191	P06396
MSF-192	MSPI-192.1	P05452
MSF-192	MSPI-192.2	O60832
MSF-193	MSPI-193	P41222
MSF-194	MSPI-194.1	P00747
MSF-194	MSPI-194.2	1160616
MSF-195	MSPI-195.1	P02790
MSF-196	MSPI-196	P01019
MSF-197	MSPI-197	P36955
MSF-198	MSPI-198	P05452
MSF-199	MSPI-199.1	P36955
MSF-199	MSPI-199.2	2745741
MSF-200	MSPI-200	P00747
MSF-201	MSPI-201	P02766
MSF-202	MSPI-202.1	P00734
MSF-202	MSPI-202.2	P51693
MSF-203	MSPI-203	P25311

MSF#	MSPI#	Accession #
MSF-205	MSPI-205	P02766
MSF-206	MSPI-206	P05090
MSF-207	MSPI-207	7662374
MSF-208	MSPI-208	Q99435
MSF-209	MSPI-209	P41222
MSF-210	MSPI-210	P36955
MSF-211	MSPI-211	P16870
MSF-212	MSPI-212.2	P02790
MSF-213	MSPI-213	P02671
MSF-215	MSPI-215	P10909
MSF-216	MSPI-216	P01028
MSF-217	MSPI-217.3	P01034
MSF-218	MSPI-218	P23142
MSF-219	MSPI-219	P00747
MSF-220	MSPI-220	P36955
MSF-221	MSPI-221.1	P02790
MSF-222	MSPI-222.1	P02679
MSF-222	MSPI-222.2	P36955
MSF-223	MSPI-223.1	P36955
MSF-223	MSPI-223.2	Q15818
MSF-224	MSPI-224.1	P02748
MSF-225	MSPI-225	P13591
MSF-226	MSPI-226	P51693
MSF-228	MSPI-228.1	P02790
MSF-228	MSPI-228.2	P04217
MSF-229	MSPI-229.1	179674
MSF-229	MSPI-229.2	P01871
MSF-230	MSPI-230	P06396
MSF-231	MSPI-231	2745741
MSF-232	MSPI-232	P05090
MSF-233	MSPI-233.1	P10909
MSF-233	MSPI-233.2	P02649
MSF-234	MSPI-234	P41222
MSF-235	MSPI-235	P16035
MSF-236	MSPI-236.1	P04217
MSF-236	MSPI-236.2	P02790
MSF-237	MSPI-237	P01034
MSF-238	MSPI-238	P01034
MSF-239	MSPI-239.1	P41222
MSF-239	MSPI-239.2	P01034
MSF-240	MSPI-240.1	P01033
MSF-240	MSPI-240.2	X57822.1
MSF-241	MSPI-241.1	179674
MSF-241	MSPI-241.2	P01871
MSF-242	MSPI-242.1	3659942
MSF-242	MSPI-242.2	P41222
MSF-243	MSPI-243	P02766
MSF-244	MSPI-244	P41222
MSF-245	MSPI-245	P01871
MSF-246	MSPI-246.1	P02766
MSF-246	MSPI-246.2	P01034

MSF#	MSPI#	Accession #
MSF-248	MSPI-248	P01028
MSF-250	MSPI-250.2	P19652
MSF-251	MSPI-251	P01024
MSF-254	MSPI-254.1	8918224
MSF-257	MSPI-257.1	1181953
MSF-257	MSPI-257.2	P02649
MSF-257	MSPI-257.3	P02766
MSF-260	MSPI-260.1	179674
MSF-260	MSPI-260.2	P01871
MSF-261	MSPI-261	P25311
MSF-262	MSPI-262.2	Q13449
MSF-265	MSPI-265	P16035
MSF-266	MSPI-266	P02649
MSF-267	MSPI-267	P09211
MSF-268	MSPI-268.1	P01034
MSF-270	MSPI-270	P02766
MSF-271	MSPI-271	U52144.1
MSF-273	MSPI-273	P02649
MSF-274	MSPI-274	P01033
MSF-276	MSPI-276	P30086
MSF-277	MSPI-277	P01871
MSF-279	MSPI-279	P41222
MSF-280	MSPI-280.1	1181953
MSF-280	MSPI-280.2	P10909
MSF-283	MSPI-283.1	P25311
MSF-283	MSPI-283.2	P01024
MSF-284	MSPI-284.1	P41222
MSF-284	MSPI-284.2	7438712
MSF-285	MSPI-285	106647
MSF-289	MSPI-289	P01019
MSF-290	MSPI-290	P41222
MSF-291	MSPI-291.1	P05155
MSF-291	MSPI-291.2	P05067
MSF-292	MSPI-292	P02766
MSF-295	MSPI-295	P05090
MSF-298	MSPI-298.1	10835792
MSF-298	MSPI-298.2	P41222
MSF-299	MSPI-299	P01024
MSF-300	MSPI-300	P07711
MSF-301	MSPI-301	P02766
MSF-302	MSPI-302	P41222
MSF-303	MSPI-303.2	P02790
MSF-305	MSPI-305	P41222
MSF-306	MSPI-306	P02790
MSF-307	MSPI-307	P01028
MSF-308	MSPI-308	P02749
MSF-311	MSPI-311.1	P01008
MSF-311	MSPI-311.2	P01876
MSF-311	MSPI-311.3	P01019
MSF-313	MSPI-313	P41222
MSF-314	MSPI-314	Q92876

MSF#	MSPI#	Accession #
MSF-316	MSPI-316	P10909
MSF-318	MSPI-318	P02766
MSF-319	MSPI-319	P04040
MSF-320	MSPI-320	P41222
MSF-322	MSPI-322	P41222
MSF-323	MSPI-323.1	P10909
MSF-323	MSPI-323.2	P02766
MSF-324	MSPI-324	P41222
MSF-325	MSPI-325.1	P01028
MSF-325	MSPI-325.2	P02750

For any MSPI, degenerate probes, or probes taken from the sequences described above by accession number may be used for screening. In the case of degenerate probes, they can be constructed from the partial amino sequence information obtained from tandem mass spectra of tryptic digest peptides of the MSPI. To screen such a gene, any probe may be used that is complementary to the gene or its complement; the probe is 10 nucleotides or longer, preferably 15 nucleotides or longer. When a library is screened, clones with insert DNA encoding the MSPI or a fragment thereof will hybridize to one or more members of the corresponding set of degenerate oligonucleotide probes (or their complement). Hybridization of such oligonucleotide probes to genomic libraries is carried out using methods known in the art. For example, hybridization with one of the above-mentioned degenerate sets of oligonucleotide probes, or their complement (or with any member of such a set, or its complement) can be performed under highly stringent or moderately stringent conditions as defined *supra*, or can be carried out in 2X SSC, 1.0% SDS at 50°C and washed using the washing conditions described *supra* for highly stringent or moderately stringent hybridization.

In yet another aspect of the invention, clones containing nucleotide sequences encoding the MSPI, MSPI fragment, MSPI-related polypeptide or the MSPI-fusion protein any of the foregoing may also be obtained by screening expression libraries. For example, DNA from the relevant source is isolated and random fragments are prepared and ligated into an expression vector (e.g., a bacteriophage, plasmid, phagemid or cosmid) such that the inserted sequence in the vector is capable of being expressed by the host cell into which the vector is then introduced. Various screening assays can then be used to select for the expressed MSPI, MSPI fragment, MSPI-related polypeptide or the MSPI-fusion protein. In one embodiment, the various anti-MSPI antibodies of the invention can be used to identify the desired clones using methods known in the art. See, for example, Harlow and Lane, 1988, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Appendix IV. Colonies or plaques from the library are brought into contact with the antibodies to identify those clones that bind antibody.

In an embodiment, colonies or plaques containing DNA that encodes MSPI, MSPI fragment, MSPI-related polypeptide or the MSPI-fusion protein can be detected using DYNA Beads according to Olsvick et al., 29th ICAAC, Houston, Tex. 1989, incorporated herein by reference. Anti-MSPI antibodies are crosslinked to tosylated DYNA Beads M280, and these antibody-containing beads are then contacted with colonies or plaques expressing recombinant polypeptides. Colonies or plaques expressing an MSPI, MSPI fragment, MSPI-related polypeptide or the MSPI-fusion protein are identified as any of those that bind the beads.

Alternatively, the anti-MSPI antibodies can be nonspecifically immobilized to a suitable support, such as silica or Celite7 resin. This material is then used to adsorb to bacterial colonies expressing the MSPI, MSPI fragment, MSPI-related polypeptide or the MSPI-fusion protein as described herein.

In another aspect, PCR amplification may be used to isolate from genomic DNA a substantially pure DNA (i.e., a DNA substantially free of contaminating nucleic acids) encoding the entire MSPI or a part thereof. Preferably such a DNA is at least 95% pure, more preferably at least 99% pure. Oligonucleotide sequences, degenerate or otherwise, that correspond to peptide sequences of MSPIs disclosed herein can be used as primers.

PCR can be carried out, e.g., by use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene Amp™ or AmpliTaq™ DNA polymerase). One can choose to synthesize several different degenerate primers, for use in the PCR reactions. It is also possible to vary the stringency of hybridization conditions used in priming the PCR reactions, to allow for greater or lesser degrees of nucleotide sequence similarity between the degenerate primers and the corresponding sequences in the DNA. After successful amplification of a segment of the sequence encoding an MSPI, that segment may be molecularly cloned and sequenced, and utilized as a probe to isolate a complete genomic clone. This, in turn, will permit the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis, as described *infra*.

The gene encoding an MSPI can also be identified by mRNA selection by nucleic acid hybridization followed by in vitro translation. In this procedure, fragments are used to isolate complementary mRNAs by hybridization. Such DNA fragments may represent available, purified DNA encoding an MSPI of another species (e.g., mouse, human). Immunoprecipitation analysis or functional assays (e.g., aggregation ability in vitro; binding to receptor) of the in vitro translation products of the isolated products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments that contain the desired sequences. In addition, specific mRNAs may be selected by adsorption of polysomes isolated from cells to immobilized antibodies that specifically recognize an MSPI. A radiolabeled cDNA encoding an MSPI can be synthesized using the selected mRNA (from the adsorbed polysomes) as a template. The radiolabeled mRNA or cDNA may then be used as a probe to identify the DNA fragments encoding an MSPI from among other genomic DNA fragments.

Alternatives to isolating genomic DNA encoding an MSPI include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or making cDNA to the mRNA which encodes the MSPI. For example, RNA for cDNA cloning of the gene encoding an MSPI can be isolated from cells which express the MSPI. Those skilled in the art will understand from the present description that other methods may be used and are within the scope of the invention.

Any suitable eukaryotic cell can serve as the nucleic acid source for the molecular cloning of the gene encoding an MSPI. The nucleic acid sequences encoding the MSPI can be isolated from vertebrate, mammalian, primate, human, porcine, bovine, feline, avian, equine, canine or murine sources. The DNA may be obtained by standard procedures known in the art from cloned DNA (e.g., a DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell. (See, e.g.,

Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II.) Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences.

The identified and isolated gene or cDNA can then be inserted into any suitable cloning vector. A large number of vector-host systems known in the art may be used. As those skilled in the art will appreciate, the only limitation is that the vector system chosen be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, plasmids such as PBR322 or pUC plasmid derivatives or the Bluescript™ vector (Stratagene) or modified viruses such as adenoviruses, adeno-associated viruses or retroviruses. The insertion into a cloning vector can be accomplished, for example, by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and the gene encoding an MSPI may be modified by homopolymeric tailing. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated gene encoding the MSPI, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The nucleotide sequences of the present invention include nucleotide sequences encoding amino acid sequences with substantially the same amino acid sequences as native MSPI, nucleotide sequences encoding amino acid sequences with functionally equivalent amino acids, nucleotide sequences encoding MSPI, MSPI fragment, MSPI-related polypeptide or the MSPI-fusion protein.

In a specific embodiment, an isolated nucleic acid molecule encoding an MSPI-related polypeptide can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of an MSPI such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Standard techniques known to those of skill in the art can be used to introduce mutations, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains

(e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed and the activity of the protein can be determined.

5.9 EXPRESSION OF DNA ENCODING MSPIs

The nucleotide sequence coding for an MSPI, MSPI fragment or MSPI-related polypeptide or other derivative of any of the foregoing, can be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational signals can also be supplied by the native gene encoding the MSPI or its flanking regions, or the native gene encoding the MSPI-related polypeptide or its flanking regions. A variety of host-vector systems may be utilized in the present invention to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used. In specific embodiments, a nucleotide sequence encoding a human gene (or a nucleotide sequence encoding a functionally active portion of a human MSPI) is expressed. In yet another embodiment, a fragment of an MSPI comprising a domain of the MSPI is expressed.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional and translational control signals and the protein coding sequences. These methods may include in vitro recombinant DNA and synthetic techniques and in vivo recombinants (genetic recombination). Expression of nucleic acid sequence encoding an MSPI or fragment thereof may be regulated by a second nucleic acid sequence so that the MSPI or fragment is expressed in a host transformed with the recombinant DNA molecule. For example, expression of an MSPI may be controlled by any promoter or enhancer element known in the art. Promoters which may be used to control the expression of the gene encoding an MSPI or an MSPI-related polypeptide include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42), the tetracycline (Tet) promoter (Gossen et al., 1995, Proc. Nat. Acad. Sci. USA 89:5547-5551); prokaryotic expression vectors such as the b-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the tac

promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25; see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94); plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella et al., Nature 303:209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner, et al., 1981, Nucl. Acids Res. 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al., 1984, Nature 310:115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286); neuronal-specific enolase (NSE) which is active in neuronal cells (Morelli et al., 1999, Gen. Virol. 80:571-83); brain-derived neurotrophic factor (BDNF) gene control region which is active in neuronal cells (Tabuchi et al., 1998, Biochem. Biophysic. Res. Com. 253:818-823); glial fibrillary acidic protein (GFAP) promoter which is active in astrocytes (Gomes et al., 1999, Braz J Med Biol Res 32(5):619-631; Morelli et al., 1999, Gen. Virol. 80:571-83) and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

In a specific embodiment, a vector is used that comprises a promoter operably linked to an MSPI-encoding nucleic acid, one or more origins of replication, and, optionally, one or more selectable markers (e.g., an antibiotic resistance gene).

In a specific embodiment, an expression construct is made by subcloning an MSPI, MSPI fragment or MSPI-related polypeptide coding sequence into the EcoRI restriction site of each of the three pGEX vectors (Glutathione S-Transferase expression vectors; Smith and Johnson, 1988, Gene 7:31-40). This allows for the expression of the MSPI product or MSPI-related polypeptide from the subclone in the correct reading frame.

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the MSPI coding sequence or MSPI-related polypeptide coding sequence may be ligated to an adenovirus

transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:355-359). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, Methods in Enzymol. 153:51-544).

Expression vectors containing inserts of a gene encoding an MSPI, MSPI fragment or MSPI-related polypeptide can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a gene encoding an MSPI inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted gene encoding an MSPI. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of a gene encoding an MSPI in the vector. For example, if the gene encoding the MSPI is inserted within the marker gene sequence of the vector, recombinants containing the gene encoding the MSPI insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the gene product (i.e., MSPI) expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the MSPI in in vitro assay systems, e.g., binding with anti-MSPI antibody.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered MSPI or MSPI-related polypeptide may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, phosphorylation of proteins). Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system will produce an unglycosylated product and expression in yeast will produce a glycosylated product. Eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, HEK293, 3T3, WI38, and in particular, neuronal cell lines such as, for example, SK-N-AS, SK-N-FI, SK-N-DZ human neuroblastomas (Sugimoto et al., 1984, J. Natl. Cancer Inst. 73: 51-57), SK-N-SH human neuroblastoma

(Biochim. Biophys. Acta, 1982, 704: 450-460), Daoy human cerebellar medulloblastoma (He et al., 1992, Cancer Res. 52: 1144-1148) DBTRG-05MG glioblastoma cells (Kruse et al., 1992, In Vitro Cell. Dev. Biol. 28A: 609-614), IMR-32 human neuroblastoma (Cancer Res., 1970, 30: 2110-2118), 1321N1 human astrocytoma (Proc. Natl Acad. Sci. USA ,1977, 74: 4816), MOG-G-CCM human astrocytoma (Br. J. Cancer, 1984, 49: 269), U87MG human glioblastoma-astrocytoma (Acta Pathol. Microbiol. Scand., 1968, 74: 465-486), A172 human glioblastoma (Olopade et al., 1992, Cancer Res. 52: 2523-2529), C6 rat glioma cells (Benda et al., 1968, Science 161: 370-371), Neuro-2a mouse neuroblastoma (Proc. Natl. Acad. Sci. USA, 1970, 65: 129-136), NB41A3 mouse neuroblastoma (Proc. Natl. Acad. Sci. USA, 1962, 48: 1184-1190), SCP sheep choroid plexus (Bolin et al., 1994, J. Virol. Methods 48: 211-221), G355-5, PG-4 Cat normal astrocyte (Haapala et al., 1985, J. Virol. 53: 827-833), Mpf ferret brain (Trowbridge et al., 1982, In Vitro 18: 952-960), and normal cell lines such as, for example, CTX TNA2 rat normal cortex brain (Radany et al., 1992, Proc. Natl. Acad. Sci. USA 89: 6467-6471) such as, for example, CRL7030 and Hs578Bst. Furthermore, different vector/host expression systems may effect processing reactions to different extents.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the MSPI may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched medium, and then are switched to a selective medium. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the MSPI. Such engineered cell lines may be particularly useful in screening and evaluation of agents that affect the endogenous activity of the MSPI.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1); and hygro, which confers resistance to hygromycin (Santerre, et al., 1984, Gene 30:147) genes.

In other specific embodiments, the MSPI, fragment, analog, or derivative may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence). For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion

proteins may facilitate purification, increase half-life in vivo, and enhance the delivery of an antigen across an epithelial barrier to the immune system. An increase in the half-life in vivo and facilitated purification has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., WO 96/22024 and WO 99/04813).

Nucleic acids encoding an MSPI, a fragment of an MSPI, an MSPI-related polypeptide, or a fragment of an MSPI-related polypeptide can fused to an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972-897).

Fusion proteins can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, a fusion protein may be made by protein synthetic techniques, e.g., by use of a peptide synthesizer.

Both cDNA and genomic sequences can be cloned and expressed.

5.10 DOMAIN STRUCTURE OF MSPIs

Domains of some MSPIs are known in the art and have been described in the scientific literature. Moreover, domains of an MSPI can be identified using techniques known to those of skill in the art. For example, one or more domains of an MSPI can be identified by using one or more of the following programs: ProDom, TMpred, and SAPS. ProDom compares the amino acid sequence of a polypeptide to a database of compiled domains (see, e.g., http://www.toulouse.inra.fr/prodom.html; Corpet F., Gouzy J. & Kahn D., 1999, Nucleic Acids Res., 27:263-267). TMpred predicts membrane-spanning regions of a polypeptide and their orientation. This program uses an algorithm that is based on the statistical analysis of TMbase, a database of naturally occuring transmembrane proteins (see, e.g., http://www.ch.embnet.org/software/TMPRED_form.html; Hofmann & Stoffel. (1993) "TMbase - A database of membrane spanning proteins segments." Biol. Chem. Hoppe-Seyler 347,166). The SAPS program analyzes polypeptides for statistically significant features like charge-clusters, repeats, hydrophobic regions, compositional domains (see, e.g., Brendel et al., 1992, Proc. Natl. Acad. Sci. USA 89: 2002-2006). Thus, based on the present description, the skilled artisan can identify domains of an MSPI having enzymatic or binding activity, and further can identify nucleotide sequences encoding such domains. These nucleotide sequences can then be used for recombinant expression of an MSPI fragment that retains the enzymatic or binding activity of the MSPI.

Based on the present description, the skilled artisan can identify domains of an MSPI having enzymatic or binding activity, and further can identify nucleotide sequences encoding such domains. These nucleotide sequences can then be used for recombinant expression of MSPI fragments that retain the enzymatic or binding activity of the MSPI.

In one embodiment, an MSPI has an amino acid sequence sufficiently similar to an identified domain of a known polypeptide. As used herein, the term "sufficiently similar" refers to a first amino acid or nucleotide sequence which contains a sufficient number of identical or equivalent (e.g., with a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have or encode a common structural domain or common functional activity or both.

An MSPI domain can be assessed for its function using techniques well known to those of skill in the art. For example, a domain can be assessed for its kinase activity or for its ability to bind to DNA using techniques known to the skilled artisan. Kinase activity can be assessed, for example, by measuring the ability of a polypeptide to phosphorylate a substrate. DNA binding activity can be assessed, for example, by measuring the ability of a polypeptide to bind to a DNA binding element in a electromobility shift assay. In a preferred embodiment, the function of a domain of an MSPI is determined using an assay described in one or more of the references identified in Table IX, infra.

5.11 PRODUCTION OF ANTIBODIES TO MSPIs

According to the invention an MSPI, MSPI fragment, MSPI-related polypeptide or the MSPI-fusion protein or derivative of any of the foregoing may be used as an immunogen to generate antibodies which immunospecifically bind such an immunogen. Such immunogens can be isolated by any convenient means, including the methods described above. Antibodies of the invention include, but are not limited to polyclonal, monoclonal, bispecific, humanized or chimeric antibodies, single chain antibodies, Fab fragments and F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds an antigen. The immunoglobulin molecules of the invention can be of any class (e.g., IgG, IgE, IgM, IgD and IgA) or subclass of immunoglobulin molecule.

In one embodiment, antibodies that recognize gene products of genes encoding MSPIs are publicly available. For example, antibodies that recognize these MSPI, MSPI fragment, MSPI-related polypeptide or the MSPI-fusion proteins include antibodies recognizing MSPI-1, MSPI-2, MSPI-3, MSPI-4, MSPI-5.1, MSPI-6, MSPI-7, MSPI-8.1, MSPI-8.2, MSPI-9, MSPI-10, MSPI-11, MSPI-12.1, MSPI-12.2, MSPI-13, MSPI-14, MSPI-15, MSPI-16, MSPI-18, MSPI-22, MSPI-24, MSPI-27, MSPI-28, MSPI-29, MSPI-30, MSPI-31, MSPI-32, MSPI-33.1, MSPI-36, MSPI-37, MSPI-39.1, MSPI-40, MSPI-42.2, MSPI-47, MSPI-48.1, MSPI-51.1, MSPI-52.1, MSPI-52.2, MSPI-54, MSPI-55, MSPI-56, MSPI-58, MSPI-60, MSPI-65, MSPI-67.1, MSPI-67.2, MSPI-70, MSPI-72, MSPI-75, MSPI-76, MSPI-78.1, MSPI-78.2, MSPI-79, MSPI-80.1, MSPI-81, MSPI-84.1, MSPI-89.1, MSPI-90, MSPI-91.1, MSPI-92, MSPI-93.1, MSPI-93.2, MSPI-95, MSPI-96, MSPI-98, MSPI-102.1, MSPI-102.2, MSPI-102.3, MSPI-104, MSPI-105.1, MSPI-105.2, MSPI-106, MSPI-107, MSPI-108, MSPI-110, MSPI-111, MSPI-113, MSPI-114, MSPI-119, MSPI-120, MSPI-122, MSPI-123.1, MSPI-127.1, MSPI-128, MSPI-130, MSPI-132, MSPI-133, MSPI-134, MSPI-137, MSPI-140, MSPI-141.2, MSPI-142, MSPI-145.2, MSPI-147.2, MSPI-149, MSPI-150, MSPI-151, MSPI-

152, MSPI-154.1, MSPI-155.2, MSPI-155.3, MSPI-156.2, MSPI-158.2, MSPI-159, MSPI-160, MSPI-161, MSPI-165.2, MSPI-167, MSPI-169.2, MSPI-170, MSPI-171, MSPI-172, MSPI-173.2, MSPI-175, MSPI-176.1, MSPI-176.2, MSPI-177, MSPI-178, MSPI-179.1, MSPI-179.2, MSPI-181.2, MSPI-182, MSPI-183, MSPI-184, MSPI-186, MSPI-187.1, MSPI-187.2, MSPI-189, MSPI-191, MSPI-192.1, MSPI-193, MSPI-194.1, MSPI-195.1, MSPI-196, MSPI-197, MSPI-198, MSPI-199.1, MSPI-199.2, MSPI-200, MSPI-201, MSPI-202.1, MSPI-205, MSPI-206, MSPI-209, MSPI-210, MSPI-212.2, MSPI-213, MSPI-215, MSPI-216, MSPI-217.3, MSPI-218, MSPI-219, MSPI-220, MSPI-221.1, MSPI-222.2, MSPI-223.1, MSPI-224.1, MSPI-228.1, MSPI-228.2, MSPI-229.2, MSPI-230, MSPI-231, MSPI-232, MSPI-233.1, MSPI-233.2, MSPI-234, MSPI-235, MSPI-236.1, MSPI-236.2, MSPI-237, MSPI-238, MSPI-239.1, MSPI-239.2, MSPI-240.1, MSPI-240.2, MSPI-241.2, MSPI-242.2, MSPI-243, MSPI-244, MSPI-245, MSPI-246.1, MSPI-246.2, MSPI-248, MSPI-251, MSPI-257.2, MSPI-257.3, MSPI-260.2, MSPI-265, MSPI-266, MSPI-268.1, MSPI-270, MSPI-273, MSPI-274, MSPI-277, MSPI-279, MSPI-280.2, MSPI-283.2, MSPI-284.1, MSPI-289, MSPI-290, MSPI-291.2, MSPI-292, MSPI-295, MSPI-298.2, MSPI-299, MSPI-301, MSPI-302, MSPI-303.2, MSPI-305, MSPI-306, MSPI-307, MSPI-311.1, MSPI-311.2, MSPI-311.3, MSPI-313, MSPI-316, MSPI-318, MSPI-319, MSPI-320, MSPI-322, MSPI-323.1, MSPI-323.2, MSPI-324, MSPI-325.1, which antibodies can be purchased from commercial sources as shown in Table VII above. In another embodiment, methods known to those skilled in the art are used to produce antibodies that recognize an MSPI, MSPI fragment, MSPI-related polypeptide or MSPI-fusion protein or derivatives of any of the foregoing.

In one embodiment of the invention, antibodies to a specific domain of an MSPI are produced. In a specific embodiment, hydrophilic fragments of an MSPI are used as immunogens for antibody production.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, e.g. ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize a specific domain of an MSPI, one may assay generated hybridomas for a product which binds to an MSPI fragment containing such domain. For selection of an antibody that specifically binds a first MSPI homolog but which does not specifically bind to (or binds less avidly to) a second MSPI homolog, one can select on the basis of positive binding to the first MSPI homolog and a lack of binding to (or reduced binding to) the second MSPI homolog. Similarly, for selection of an antibody that specifically binds an MSPI but which does not specifically bind to (or binds less avidly to) a different isoform of the same protein (such as a different glycoform having the same core peptide as the MSPI), one can select on the basis of positive binding to the MSPI and a lack of binding to (or reduced binding to) the different isoform (e.g., a different glycoform). Thus, the present invention provides an antibody (preferably a monoclonal antibody) that binds with greater affinity (preferably at least 2-fold, more preferably at least 5-fold still more preferably at least 10-fold greater affinity) to an MSPI than to a different isoform or isoforms (e.g., glycoforms) of the MSPI.

Polyclonal antibodies which may be used in the methods of the invention are heterogeneous populations of antibody molecules derived from the sera of immunized animals. Unfractionated immune serum can also be used. Various procedures known in the art may be used for the production of polyclonal antibodies to an MSPI, MSPI fragment, MSPI-related

polypeptide or the MSPI-fusion protein. In a particular embodiment, rabbit polyclonal antibodies to an epitope of an MSPI, MSPI fragment, MSPI-related polypeptide or the MSPI-fusion protein can be obtained. For example, for the production of polyclonal or monoclonal antibodies, various host animals can be immunized by injection with the native or a synthetic (e.g., recombinant) version of an MSPI, MSPI fragment, MSPI-related polypeptide or the MSPI-fusion protein, including but not limited to rabbits, mice, rats, etc. The Preferred Technology described herein provides isolated MSPIs suitable for such immunization. If the MSPI is purified by gel electrophoresis, the MSPI can be used for immunization with or without prior extraction from the polyacrylamide gel. Various adjuvants may be used to enhance the immunological response, depending on the host species, including, but not limited to, complete or incomplete Freund's adjuvant, a mineral gel such as aluminum hydroxide, surface active substance such as lysolecithin, pluronic polyol, a polyanion, a peptide, an oil emulsion, keyhole limpet hemocyanin, dinitrophenol, and an adjuvant such as BCG (bacille Calmette-Guerin) or corynebacterium parvum. Additional adjuvants are also well known in the art.

For preparation of monoclonal antibodies (mAbs) directed toward an MSPI, MSPI fragment, MSPI-related polypeptide or the MSPI-fusion protein, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAbs of the invention may be cultivated in vitro or in vivo. In an additional embodiment of the invention, mAbs can be produced in germ-free animals utilizing known technology (PCT/US90/02545).

The mAbs include but are not limited to human mAbs and chimeric mAbs (e.g., human-mouse chimeras). A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a human immunoglobulin constant region and a variable region derived from a murine mAb. (See, e.g., US 4,816,567 and U.S. Patent No. 4,816397.) Humanized antibodies are antibody molecules from non-human species having one or more complementarily determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule. (See, e.g US 5,585,089, which is incorporated herein by reference in its entirety.)

Chimeric and humanized mAbs can be produced by recombinant DNA techniques known in the art, for example using methods described in WO 87/02671; EP 184,187A; EP 171,496A; EP 173,494A; WO 86/01533; US 4,816,567; EP 125,023A; Better et al., 1988, Science 240:1041-1043; Liu et al., 1987, Proc. Natl. Acad. Sci. USA 84:3439-3443; Liu et al., 1987, J. Immunol. 139:3521-3526; Sun et al., 1987, Proc. Natl. Acad. Sci. USA 84:214-218; Nishimura et al., 1987, Canc. Res. 47:999-1005; Wood et al., 1985, Nature 314:446-449; and Shaw et al., 1988, J. Natl. Cancer Inst. 80:1553-1559; Morrison, 1985, Science 229:1202-1207; Oi et al., 1986, Bio/Techniques 4:214; U.S. 5,225,539; Jones et al., 1986, Nature 321:552-525; Verhoeyan et al. (1988) Science 239:1534; and Beidler et al., 1988, J. Immunol. 141:4053-4060.

Completely human antibodies are particularly desirable for therapeutic treatment of human subjects. Such antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of an MSPI of the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,569,825; U.S. Patent 5,661,016; and U.S. Patent 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al. (1994) Bio/technology 12:899-903).

The antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT/GB91/01134; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired

host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988).

The invention further provides for the use of bispecific antibodies, which can be made by methods known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Milstein et al., 1983, Nature 305:537-539). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker et al., 1991, EMBO J. 10:3655-3659.

According to a different and preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details for generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology,1986, 121:210.

The invention provides functionally active fragments, derivatives or analogs of the anti-MSPI immunoglobulin molecules. Functionally active means that the fragment, derivative

or analog is able to elicit anti-anti-idiotype antibodies (i.e., tertiary antibodies) that recognize the same antigen that is recognized by the antibody from which the fragment, derivative or analog is derived. Specifically, in a preferred embodiment the antigenicity of the idiotype of the immunoglobulin molecule may be enhanced by deletion of framework and CDR sequences that are C-terminal to the CDR sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any binding assay method known in the art.

The present invention provides antibody fragments such as, but not limited to, F(ab')2 fragments and Fab fragments. Antibody fragments which recognize specific epitopes may be generated by known techniques. F(ab')2 fragments consist of the variable region, the light chain constant region and the CH1 domain of the heavy chain and are generated by pepsin digestion of the antibody molecule. Fab fragments are generated by reducing the disulfide bridges of the F(ab')2 fragments. The invention also provides heavy chain and light chain dimers of the antibodies of the invention, or any minimal fragment thereof such as Fvs or single chain antibodies (SCAs) (e.g., as described in U.S. 4,946,778; Bird, 1988, Science 242:423-42; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989; Nature 334:544-54), or any other molecule with the same specificity as the antibody of the invention. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may be used (Skerra et al., 1988, Science 242:1038-1041).

In other embodiments, the invention provides fusion proteins of the immunoglobulins of the invention (or functionally active fragments thereof), for example in which the immunoglobulin is fused via a covalent bond (e.g., a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably at least 10, 20 or 50 amino acid portion of the protein) that is not the immunoglobulin. Preferably the immunoglobulin, or fragment thereof, is covalently linked to the other protein at the N-terminus of the constant domain. As stated above, such fusion proteins may facilitate purification, increase half-life in vivo, and enhance the delivery of an antigen across an epithelial barrier to the immune system.

The immunoglobulins of the invention include analogs and derivatives that are either modified, i.e, by the covalent attachment of any type of molecule as long as such covalent attachment that does not impair immunospecific binding. For example, but not by way of limitation, the derivatives and analogs of the immunoglobulins include those that have been further modified, e.g., by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, etc. Additionally, the analog or derivative may contain one or more non-classical amino acids.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the MSPIs of the invention, e.g., for imaging these polypeptides, measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc.

5.12 EXPRESSION OF ANTIBODIES

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression techniques.

Recombinant expression of antibodies, or fragments, derivatives or analogs thereof, requires construction of a nucleic acid that encodes the antibody. If the nucleotide sequence of the antibody is known, a nucleic acid encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., 1994, BioTechniques 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, the nucleic acid encoding the antibody may be obtained by cloning the antibody. If a clone containing the nucleic acid encoding the particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the antibody may be obtained from a suitable source (e.g., an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

If an antibody molecule that specifically recognizes a particular antigen is not available (or a source for a cDNA library for cloning a nucleic acid encoding such an antibody), antibodies specific for a particular antigen may be generated by any method known in the art, for example, by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating mAbs. Alternatively, a clone encoding at least the Fab portion of the antibody may be obtained by screening Fab expression libraries (e.g., as described in Huse et al., 1989, Science 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (See, e.g., Clackson et al., 1991, Nature 352:624; Hane et al., 1997 Proc. Natl. Acad. Sci. USA 94:4937).

Once a nucleic acid encoding at least the variable domain of the antibody molecule is obtained, it may be introduced into a vector containing the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., WO 86/05807; WO 89/01036; and U.S. 5,122,464). Vectors containing the complete light or heavy chain for co-expression with the nucleic acid to allow the expression of a complete antibody molecule are also available. Then, the nucleic acid encoding the antibody can be used to introduce the nucleotide substitution(s) or deletion(s) necessary to substitute (or delete) the one or more variable region cysteine residues participating in an intrachain disulfide bond with an amino acid residue that does not contain a sulfhydyl group. Such modifications can be carried out by any method known in the art for the introduction of specific mutations or deletions in a nucleotide sequence, for example, but not limited to, chemical mutagenesis, in vitro site directed mutagenesis (Hutchinson et al., 1978, J. Biol. Chem. 253:6551), PCR based methods, etc.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. 81:851-855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a

chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human antibody constant region, e.g., humanized antibodies.

Once a nucleic acid encoding an antibody molecule of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the protein of the invention by expressing nucleic acid containing the antibody molecule sequences are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing an antibody molecule coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. See, for example, the techniques described in Sambrook et al. (1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and Ausubel et al. (eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY).

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention.

The host cells used to express a recombinant antibody of the invention may be either bacterial cells such as *Escherichia coli*, or, preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule. In particular, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., 198, Gene 45:101; Cockett et al., 1990, Bio/Technology 8:2).

A variety of host-expression vector systems may be utilized to express an antibody molecule of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli, B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces, Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, HEK293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example,

when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions comprising an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). In mammalian host cells, a number of viral-based expression systems (e.g., an adenovirus expression system) may be utilized.

As discussed above, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein.

For long-term, high-yield production of recombinant antibodies, stable expression is preferred. For example, cell lines that stably express an antibody of interest can be produced by transfecting the cells with an expression vector comprising the nucleotide sequence of the antibody and the nucleotide sequence of a selectable marker (e.g., neomycin or hygromycin), and selecting for expression of the selectable marker. Such engineered cell lines may be particularly useful in screening and evaluation of agents that interact directly or indirectly with the antibody molecule.

The expression levels of the antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., 1983, Mol. Cell. Biol. 3:257).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy

chain (Proudfoot, 1986, Nature 322:52; Kohler, 1980, Proc. Natl. Acad. Sci. USA 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once the antibody molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification of an antibody molecule, for example, by chromatography (e.g., ion exchange chromatography, affinity chromatography such as with protein A or specific antigen, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972-897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni2+ nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

5.13 CONJUGATED ANTIBODIES

In a preferred embodiment, anti-MSPI antibodies or fragments thereof are conjugated to a diagnostic or therapeutic moiety. The antibodies can be used for diagnosis or to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include 125I, 131I, 111In and 99Tc.

An anti-MSPI antibodies or fragments thereof can be conjugated to a therapeutic agent or drug moiety to modify a given biological response. The therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents, e.g., small molecules. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha -interferon, beta -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth

factor.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described in U.S. 4,676,980.

An antibody with or without a therapeutic moiety conjugated to it can be used as a therapeutic that is administered alone or in combination with cytokine(s).

5.14 DIAGNOSIS OF MS

In accordance with the present invention, a first sample of CSF, serum, plasma or urine obtained from a subject suspected of having or known to have MS can be used for diagnosis or monitoring. In one embodiment, a decreased abundance of one or more MSFs or MSPIs (or any combination of them) in a first sample or sample set relative to a second sample or sample set (from a subject or subjects free from MS) or a previously determined reference range indicates the presence of MS; MSFs and MSPIs suitable for this purpose are identified in Tables I and IV, respectively, as described in detail above. In another embodiment of the invention, an increased abundance of one or more MSFs or MSPIs (or any combination of them) in a first sample or sample set compared to a second sample or sample set or a previously determined reference range indicates the presence of MS; MSFs and MSPIs suitable for this purpose are identified in Tables II and V, respectively, as described in detail above. In another embodiment, the relative abundance of one or more MSFs or MSPIs (or any combination of them) in a first sample or sample set compared to a second sample or sample set or a previously determined reference range indicates a subtype of MS (e.g., benign or progressive MS). In yet another embodiment, the relative abundance of one or more MSFs or MSPIs (or any combination of them) in a first sample or sample set relative to a second sample or sample set or a previously determined reference range indicates the degree or severity of MS. In any of the aforesaid methods, detection of one or more MSPIs described herein may optionally be combined with detection of one or more additional biomarkers for MS including, but not limited to, oligoclonal immunoglobulin bands in CSF revealed by isoelectric focusing (Reiber H et al. (1998) Mult Scler 3: 111-7). Any suitable method in the art can be employed to measure the level of MSFs and MSPIs, including but not limited to the Preferred Technology described herein, kinase assays, immunoassays to detect and/or visualize the MSPIs (e.g., Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunocytochemistry, etc.). In cases where an MSPI has

a known function, an assay for that function may be used to measure MSPI expression. In a further embodiment, a decreased abundance of mRNA including one or more MSPIs identified in Table IV (or any combination of them) in a first sample or sample set relative to a second sample or sample set or a previously determined reference range indicates the presence of MS. In yet a further embodiment, an increased abundance of mRNA encoding one or more MSPIs identified in Table V (or any combination of them) in a first sample or sample set relative to a second sample or sample set or previously determined reference range indicates the presence of MS. Any suitable hybridization assay can be used to detect MSPI expression by detecting and/or visualizing mRNA encoding the MSPI (e.g., Northern assays, dot blots, in situ hybridization, etc.).

In another embodiment of the invention, labeled antibodies, derivatives and analogs thereof, which specifically bind to an MSPI can be used for diagnostic purposes to detect, diagnose, or monitor MS. Preferably, MS is detected in an animal, more preferably in a mammal and most preferably in a human.

5.15 SCREENING ASSAYS

The invention provides methods for identifying agents (e.g., candidate agents) that bind to an MSPI or have a stimulatory or inhibitory effect on the expression or activity of an MSPI. The invention also provides methods of identifying agents or candidate agents that bind to an MSPI fragment, MSPI-related polypeptide or the MSPI-fusion protein or have a stimulatory or inhibitory effect on the expression or activity of an MSPI fragment, MSPI-related polypeptide or an MSPI fusion protein. Examples of agents or candidate agents include, but are not limited to, nucleic acids (e.g., DNA and RNA), carbohydrates, lipids, proteins, peptides, peptidomimetics, small molecules and other drugs. Agents can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, 1997, Anticancer Drug Des. 12: 145; U.S. 5,738,996; and U.S. 5,807,683).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al., 1993, Proc. Natl. Acad. Sci. USA 90:6909; Erb et al., 1994, Proc. Natl. Acad. Sci. USA 91:11422; Zuckermann et al., 1994, J. Med. Chem. 37:2678; Cho et al., 1993, Science 261:1303; Carrell et al., 1994, Angew. Chem. Int. Ed. Engl. 33:2059; Carell et al., 1994, Angew. Chem. Int. Ed. Engl. 33:2061; and Gallop et al., 1994; J. Med. Chem. 37:1233.

Libraries of compounds may be presented, e.g., presented in solution (e.g., Houghten, 1992, Bio/Techniques 13:412-421), or on beads (Lam, 1991, Nature 354:82-84), chips (Fodor, 1993, Nature 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (US Patent Nos. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull et al., 1992, Proc. Natl. Acad. Sci. USA 89:1865-1869) or phage (Scott and Smith, 1990, Science 249:386-390; Devlin, 1990, Science 249:404-406; Cwirla et al., 1990, Proc. Natl. Acad. Sci. USA 87:6378-6382; and Felici, 1991, J. Mol. Biol. 222:301-310), each of which is incorporated herein in its entirety by reference.

In one embodiment, agents that interact with (i.e., bind to) an MSPI, an MSPI fragment (e.g. a functionally active fragment), an MSPI-related polypeptide, a fragment of an MSPI-related polypeptide, or an MSPI fusion protein are identified in a cell-based assay system. In accordance with this embodiment, cells expressing an MSPI, a fragment of an MSPI, an MSPI-related polypeptide, a fragment of an MSPI-related polypeptide, or an MSPI fusion protein are contacted with a candidate agent or a control agent and the ability of the candidate agent to interact with the MSPI is determined. If desired, this assay may be used to screen a plurality (e.g. a library) of candidate agents. The cell, for example, can be of prokaryotic origin (e.g., *E. coli*) or eukaryotic origin (e.g., yeast or mammalian). Further, the cells can express the MSPI, fragment of the MSPI, MSPI-related polypeptide, a fragment of the MSPI-related polypeptide, or an MSPI fusion protein endogenously or be genetically engineered to express the MSPI, fragment of the MSPI, MSPI-related polypeptide, a fragment of the MSPI-related polypeptide, or an MSPI fusion protein. In certain instances, the MSPI, fragment of the MSPI, MSPI-related polypeptide, a fragment of the MSPI-related polypeptide, or an MSPI fusion protein or the candidate agent is labeled, for example with a radioactive label (such as 32P, 35S or 125I) or a fluorescent label (such as fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde or fluorescamine) to enable detection of an interaction between an MSPI and a candidate agent. The ability of the candidate agent to interact directly or indirectly with an MSPI, a fragment of an MSPI, an MSPI-related polypeptide, a fragment of an MSPI-related polypeptide, or an MSPI fusion protein can be determined by methods known to those of skill in the art. For example, the interaction between a candidate agent and an MSPI, a fragment of an MSPI, an MSPI-related polypeptide, a fragment of an MSPI-related polypeptide, or an MSPI fusion protein can be determined by flow cytometry, a scintillation assay, immunoprecipitation or western blot analysis.

In another embodiment, agents that interact with (i.e., bind to) an MSPI, an MSPI fragment (e.g., a functionally active fragment) an MSPI-related polypeptide, a fragment of an MSPI-related polypeptide, or an MSPI fusion protein are identified in a cell-free assay system. In accordance with this embodiment, a native or recombinant MSPI or fragment thereof, or a native or recombinant MSPI-related polypeptide or fragment thereof, or an MSPI-fusion protein or fragment thereof, is contacted with a candidate agent or a control agent and the ability of the candidate agent to interact with the MSPI or MSPI-related polypeptide, or MSPI fusion protein is determined. If desired, this assay may be used to screen a plurality (e.g. a library) of candidate agents. Preferably, the MSPI, MSPI fragment, MSPI-related polypeptide, a fragment of an MSPI-related polypeptide, or an MSPI-fusion protein is first immobilized, by, for example, contacting the MSPI, MSPI fragment, MSPI-related polypeptide, a fragment of an MSPI-related polypeptide, or an MSPI fusion protein with an immobilized antibody which specifically recognizes and binds it, or by contacting a purified preparation of the MSPI, MSPI fragment, MSPI-related polypeptide, fragment of an MSPI-related polypeptide, or an MSPI fusion protein with a surface designed to bind proteins. The MSPI, MSPI fragment, MSPI-related polypeptide, a fragment of an MSPI-related polypeptide, or an MSPI fusion protein may be partially or completely purified (e.g., partially or completely free of other polypeptides) or part of a cell lysate. Further, the MSPI, MSPI fragment, MSPI-related polypeptide, a fragment of an MSPI-related polypeptide may be a fusion protein comprising the MSPI or a

biologically active portion thereof, or MSPI-related polypeptide and a domain such as glutathionine-S-transferase. Alternatively, the MSPI, MSPI fragment, MSPI-related polypeptide, fragment of an MSPI-related polypeptide or MSPI fusion protein can be biotinylated using techniques well known to those of skill in the art (e.g., biotinylation kit, Pierce Chemicals; Rockford, IL). The ability of the candidate agent to interact with an MSPI, MSPI fragment, MSPI-related polypeptide, a fragment of an MSPI-related polypeptide, or an MSPI fusion protein can be can be determined by methods known to those of skill in the art.

In another embodiment, a cell-based assay system is used to identify agents that bind to or modulate the activity of a protein, such as an enzyme, or a biologically active portion thereof, which is responsible for the production or degradation of an MSPI or is responsible for the post- translational modification of an MSPI. In a primary screen, a plurality (e.g., a library) of candidate agents are contacted with cells that naturally or recombinantly express: (i) an MSPI, an MSPI homolog an MSPI-related polypeptide, an MSPI fusion protein, or a biologically active fragment of any of the foregoing; and (ii) a protein that is responsible for processing of the MSPI, MSPI homolog, MSPI-related polypeptide, MSPI fusion protein, or fragment in order to identify agents that modulate the production, degradation, or post-translational modification of the MSPI, MSPI homolog, MSPI-related polypeptide, MSPI fusion protein or fragment. If desired, candidate agents identified in the primary screen can then be assayed in a secondary screen against cells naturally or recombinantly expressing the specific MSPIs of interest. The ability of the candidate agent to modulate the production, degradation or post-translational modification of an MSPI, homolog, MSPI-related polypeptide, or MSPI fusion protein can be determined by methods known to those of skill in the art, including without limitation, flow cytometry, a scintillation assay, immunoprecipitation and western blot analysis.

In another embodiment, agents that competitively interact with (i.e., bind to) an MSPI, MSPI fragment, MSPI-related polypeptide, a fragment of an MSPI-related polypeptide, or an MSPI fusion protein are identified in a competitive binding assay. In accordance with this embodiment, cells expressing an MSPI, MSPI fragment, MSPI-related polypeptide, a fragment of an MSPI-related polypeptide, or an MSPI fusion protein are contacted with a candidate agent and a agent known to interact with the MSPI, MSPI fragment, MSPI-related polypeptide, a fragment of an MSPI-related polypeptide or an MSPI fusion protein; the ability of the candidate agent to competitively interact with the MSPI, MSPI fragment, MSPI-related polypeptide, fragment of an MSPI-related polypeptide, or an MSPI fusion protein is then determined. Alternatively, agents that competitively interact with (i.e., bind to) an MSPI, MSPI fragment, MSPI-related polypeptide or fragment of an MSPI-related polypeptide are identified in a cell-free assay system by contacting an MSPI, MSPI fragment, MSPI-related polypeptide, fragment of an MSPI-related polypeptide, or an MSPI fusion protein with a candidate agent and a agent known to interact with the MSPI, MSPI-related polypeptide or MSPI fusion protein. As stated above, the ability of the candidate agent to interact with an MSPI, MSPI fragment, MSPI-related polypeptide, a fragment of an MSPI-related polypeptide, or an MSPI fusion protein can be determined by methods known to those of skill in the art. These assays, whether cell-based or cell-free, can be used to screen a plurality (e.g., a library) of candidate agents.

In another embodiment, agents that modulate (i.e., upregulate or downregulate) the

expression of an MSPI, or an MSPI-related polypeptide are identified by contacting cells (e.g., cells of prokaryotic origin or eukaryotic origin) expressing the MSPI, or MSPI-related polypeptide with a candidate agent or a control agent (e.g., phosphate buffered saline (PBS)) and determining the expression of the MSPI, MSPI-related polypeptide, or MSPI fusion protein, mRNA encoding the MSPI, or mRNA encoding the MSPI-related polypeptide. The level of expression of a selected MSPI, MSPI-related polypeptide, mRNA encoding the MSPI, or mRNA encoding the MSPI-related polypeptide in the presence of the candidate agent is compared to the level of expression of the MSPI, MSPI-related polypeptide, mRNA encoding the MSPI, or mRNA encoding the MSPI-related polypeptide in the absence of the candidate agent (e.g., in the presence of a control agent). The candidate agent can then be identified as a modulator of the expression of the MSPI, or an MSPI-related polypeptide based on this comparison. For example, when expression of the MSPI or mRNA is significantly greater in the presence of the candidate agent than in its absence, the candidate agent is identified as a stimulator of expression of the MSPI or mRNA. Alternatively, when expression of the MSPI or mRNA is significantly less in the presence of the candidate agent than in its absence, the candidate agent is identified as an inhibitor of the expression of the MSPI or mRNA. The level of expression of an MSPI or the mRNA that encodes it can be determined by methods known to those of skill in the art. For example, mRNA expression can be assessed by Northern blot analysis or RT-PCR, and protein levels can be assessed by western blot analysis.

The candidate agent may be an agonist or an antagonist of the MSPI, MSPI-related polypeptide, or MSPI fusion protein, or of an upstream effector of the MSPI, MSPI-related polypeptide or MSPI fusion protein.

In another embodiment, agents that modulate the activity of an MSPI, or an MSPI-related polypeptide are identified by contacting a preparation containing the MSPI or MSPI-related polypeptide, or cells (e.g., prokaryotic or eukaryotic cells) expressing the MSPI or MSPI-related polypeptide with a candidate agent or a control agent and determining the ability of candidate agent to modulate (e.g., stimulate or inhibit) the activity of the MSPI or MSPI-related polypeptide. The activity of an MSPI or an MSPI-related polypeptide can be assessed by detecting changes in a downstream effector such as, without limitation, induction of a cellular signal transduction pathway of the MSPI or MSPI-related polypeptide (e.g., intracellular $Ca2^{+}$, diacylglycerol, IP3, etc.), detecting catalytic or enzymatic activity of the target on a suitable substrate, detecting the induction of a reporter gene (e.g., a regulatory element that is responsive to an MSPI or an MSPI-related polypeptide and is operably linked to a nucleic acid encoding a detectable marker, e.g., luciferase), or detecting a cellular response, for example, cellular differentiation, or cell proliferation. Based on the present description, techniques known to those of skill in the art can be used for measuring these activities (see, e.g., U.S. Patent No. 5,401,639, which is incorporated herein by reference). The candidate agent can then be identified as a modulator of the activity of an MSPI or MSPI-related polypeptide by comparing the effects of the candidate agent to the control agent. Suitable control agents include phosphate buffered saline (PBS) and normal saline (NS).

In another embodiment, agents that modulate (i.e., upregulate or downregulate) the expression, activity or both the expression and activity of an MSPI or MSPI-related polypeptide are identified in an animal model. Examples of suitable animals include, but are not limited to, mice, rats, rabbits, monkeys, guinea pigs, dogs and cats. Preferably, the animal

used represent a model of MS (e.g., experimental autoimmune encephalomyelitis (EAE) (Steinman (1999) Neuron, 24:511-514)). In accordance with this embodiment, the candidate agent or a control agent is administered (e.g., orally, rectally or parenterally such as intraperitoneally or intravenously) to a suitable animal and the effect on the expression, activity or both expression and activity of the MSPI or MSPI-related polypeptide is determined. Changes in the expression of an MSPI or MSPI-related polypeptide can be assessed by the methods outlined above.

In yet another embodiment, an MSPI or MSPI-related polypeptide is used as a "bait protein" in a two-hybrid assay or three hybrid assay to identify other proteins that bind to or interact with an MSPI or MSPI-related polypeptide (see, e.g., U.S. Patent No. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-12054; Bartel et al. (1993) Bio/Techniques 14:920-924; Iwabuchi et al. (1993) Oncogene 8:1693-1696; and WO 94/10300). As those skilled in the art will appreciate, such binding proteins may be involved in the propagation of signals by the MSPIs of the invention as, for example, upstream or downstream elements of a signaling pathway involving the MSPIs of the invention.

Table IX enumerates scientific publications describing suitable assays for detecting or quantifying enzymatic or binding activity of an MSPI, an MSPI analog, an MSPI-related polypeptide, or a fragment of any of the foregoing. Each such reference is hereby incorporated in its entirety. In a preferred embodiment, as assay referenced in Table IX is used in the screens and assays described herein, for example to screen for or identify candidate agent that modulates the activity and or expression of an MSPI, MSPI analog, or MSPI-related polypeptide, a fragment of any of the foregoing.

Table IX.

MSPI	References
MSPI-81	*Nature Structural Biology* 7, 312-321, 2000
MSPI-133	*J. Am. Chem. Soc.* 122, 2178-2192, 2000
MSPI-187.2	
MSPI-201	
MSPI-205	
MSPI-243	
MSPI-246.1	
MSPI-257.3	
MSPI-270	
MSPI-292	
MSPI-301	
MSPI-318	
MSPI-323.2	

MSPI	References
MSPI-8.2 MSPI-52.1 MSPI-52.2 MSPI-76 MSPI-93.1 MSPI-102.1 MSPI-128 MSPI-171 MSPI-233.2 MSPI-251 MSPI-266 MSPI-273 MSPI-283.2 MSPI-323.3	Clin Chem 1993 Feb 39:2 309-12 J Immunol Methods 1987 Aug 24 102:1 7-14
MSPI-14 MSPI-30 MSPI-84.1 MSPI-216 MSPI-244 MSPI-307 MSPI-325.1	J Clin Lab Immunol 1986 Dec 21:4 201-7
MSPI-7 MSPI-127.1 MSPI-137 MSPI-142 MSPI-161 MSPI-182 MSPI-196 MSPI-289 MSPI-311.3	Neuroendocrinology 1992 Mar 55:3 308-16
MSPI-91.2	J Chromatogr 1991 Jul 5 567:2 369-80; Clin Chem 1989 Apr 35:4 582-6
MSPI-300	Eur J Biochem 2000 267:2965-2972 Eur J Biochem 2000 267:5085-5092 Eur J Biochem 2000 267:6311-6318

This invention further provides novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

5.16 THERAPEUTIC USES OF MSPIs

The invention provides for treatment or prevention of various diseases and disorders by administration of a therapeutic agent. Such agents include but are not limited to: MSPIs, MSPI analogs, MSPI-related polypeptides and derivatives (including fragments) thereof;

antibodies to the foregoing; nucleic acids encoding MSPIs, MSPI analogs, MSPI-related polypeptides and fragments thereof; antisense nucleic acids to a gene encoding an MSPI or MSPI-related polypeptide; and modulator (e.g., agonists and antagonists) of a gene encoding an MSPI or MSPI-related polypeptide. An important feature of the present invention is the identification of genes encoding MSPIs involved in MS. MS can be treated (e.g. to ameliorate symptoms or to retard onset or progression) or prevented by administration of a therapeutic agent that promotes function or expression of one or more MSPIs that are decreased in the CSF of MS subjects having MS, or by administration of a therapeutic agent that reduces function or expression of one or more MSPIs that are increased in the CSF of subjects having MS.

In one embodiment, one or more antibodies each specifically binding to an MSPI are administered alone or in combination with one or more additional therapeutic agents or treatments. Examples of such therapeutic agents or treatments include, but are not limited to, Interferon β-1b (Betaseron™, Betaferon™), Interferon β-1a (Avonex™, Rebif™), Glatiramer acetate (Copaxone™), intravenous immunoglobulin and, for incidences of acute relapse, therapies with corticosteroids (Noseworthy (1999) Nature 399:suppl. A40-A47).

Preferably, a biological product such as an antibody is allogeneic to the subject to which it is administered. In a preferred embodiment, a human MSPI or a human MSPI-related polypeptide, a nucleotide sequence encoding a human MSPI or a human MSPI-related polypeptide, or an antibody to a human MSPI or a human MSPI-related polypeptide, is administered to a human subject for therapy (e.g. to ameliorate symptoms or to retard onset or progression) or prophylaxis.

5.16.1 Treatment And Prevention of MS

MS is treated or prevented by administration to a subject suspected of having or known to have MS or to be at risk of developing MS of a agent that modulates (i.e., increases or decreases) the level or activity (i.e., function) of one or more MSPIs -- or the level of one or more MSFs - that are differentially present in the CSF of subjects having MS compared with CSF of subjects free from MS. In one embodiment, MS is treated or prevented by administering to a subject suspected of having or known to have MS or to be at risk of developing MS a agent that upregulates (i.e., increases) the level or activity (i.e., function) of one or more MSPIs - or the level of one or more MSFs - that are decreased in the CSF of subjects having MS. In another embodiment, a agent is administered that upregulates the level or activity (i.e., function) of one or more MSPIs - or the level of one or more MSFs - that are increased in the CSF of subjects having MS. Examples of such a agent include but are not limited to: MSPIs, MSPI fragments and MSPI-related polypeptides; nucleic acids encoding an MSPI, an MSPI fragment and an MSPI-related polypeptide (e.g., for use in gene therapy); and, for those MSPIs or MSPI-related polypeptides with enzymatic activity, agents or molecules known to modulate that enzymatic activity. Other agents that can be used, e.g., MSPI agonists, can be identified using in vitro assays.

MS is also treated or prevented by administration to a subject suspected of having or known to have MS or to be at risk of developing MS of a agent that downregulates the level or activity of one or more MSPIs - or the level of one or more MSFs - that are increased in the CSF of subjects having MS. In another embodiment, a agent is administered that downregulates the level or activity of one or more MSPIs - or the level of one or more MSFs -

that are decreased in the CSF of subjects having MS. Examples of such a agent include, but are not limited to, MSPI antisense oligonucleotides, ribozymes, antibodies directed against MSPIs, and agents that inhibit the enzymatic activity of an MSPI. Other useful agents e.g., MSPI antagonists and small molecule MSPI antagonists, can be identified using in vitro assays.

In a preferred embodiment, therapy or prophylaxis is tailored to the needs of an individual subject. Thus, in specific embodiments, agents that promote the level or function of one or more MSPIs, or the level of one or more MSFs, are therapeutically or prophylactically administered to a subject suspected of having or known to have MS, in whom the levels or functions of said one or more MSPIs, or levels of said one or more MSFs, are absent or are decreased relative to a control or normal reference range. In further embodiments, agents that promote the level or function of one or more MSPIs, or the level of one or more MSFs, are therapeutically or prophylactically administered to a subject suspected of having or known to have MS in whom the levels or functions of said one or more MSPIs, or levels of said one or more MSFs, are increased relative to a control or to a reference range. In further embodiments, agents that decrease the level or function of one or more MSPIs, or the level of one or more MSFs, are therapeutically or prophylactically administered to a subject suspected of having or known to have MS in whom the levels or functions of said one or more MSPIs, or levels of said one or more MSFs, are increased relative to a control or to a reference range. In further embodiments, agents that decrease the level or function of one or more MSPIs, or the level of one or more MSFs, are therapeutically or prophylactically administered to a subject suspected of having or known to have MS in whom the levels or functions of said one or more MSPIs, or levels of said one or more MSFs, are decreased relative to a control or to a reference range. The change in MSPI function or level, or MSF level, due to the administration of such agents can be readily detected, e.g., by obtaining a sample (e.g., a sample of CSF, blood or urine or a tissue sample such as biopsy tissue) and assaying in vitro the levels of said MSFs or the levels or activities of said MSPIs, or the levels of mRNAs encoding said MSPIs. or any combination of the foregoing. Such assays can be performed before and after the administration of the agent as described herein.

The agents of the invention include but are not limited to any agent, e.g., a small organic molecule, protein, peptide, antibody, nucleic acid, etc. that restores the MS MSPI or MSF profile towards normal with the proviso that such agents do not include Interferon β-1b (Betaseron™, Betaferon™), Interferon β-1a (Avonex™, Rebif™), Glatiramer acetate (Copaxone™), intravenous immunoglobulin and, for incidences of acute relapse, therapies with corticosteroids (Noseworthy (1999) Nature 399:suppl. A40-A47).

5.16.2 Gene Therapy

In a specific embodiment, nucleic acids comprising a sequence encoding an MSPI, an MSPI fragment, MSPI-related polypeptide or fragment of an MSPI-related polypeptide, are administered to promote MSPI function by way of gene therapy. Gene therapy refers to administration to a subject of an expressed or expressible nucleic acid. In this embodiment, the nucleic acid produces its encoded polypeptide that mediates a therapeutic effect by promoting MSPI function.

Any of the methods for gene therapy available in the art can be used according to the

present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

In a preferred aspect, the agent comprises a nucleic acid encoding an MSPI or fragment or chimeric protein thereof, said nucleic acid being part of an expression vector that expresses an MSPI or fragment or chimeric protein thereof in a suitable host. In particular, such a nucleic acid has a promoter operably linked to the MSPI coding region, said promoter being inducible or constitutive (and, optionally, tissue-specific). In another particular embodiment, a nucleic acid molecule is used in which the MSPI coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the MSPI nucleic acid (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

Delivery of the nucleic acid into a subject may be direct, in which case the subject is directly exposed to the nucleic acid or nucleic acid-carrying vector; this approach is known as in vivo gene therapy. Alternatively, delivery of the nucleic acid into the subject may be indirect, in which case cells are first transformed with the nucleic acid in vitro and then transplanted into the subject; this approach is known as ex vivo gene therapy.

In a specific embodiment, the nucleic acid is directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by infection using a defective or attenuated retroviral or other viral vector (see U.S. Patent No. 4,980,286); by direct injection of naked DNA; by use of microparticle bombardment (e.g., a gene gun; Biolistic™, Dupont); by coating with lipids, cell-surface receptors or transfecting agents; by encapsulation in liposomes, microparticles or microcapsules; by administering it in linkage to a peptide which is known to enter the nucleus; or by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), which can be used to target cell types specifically expressing the receptors. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., WO 92/06180, WO 92/22635, WO92/20316, WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

In a specific embodiment, a viral vector that contains a nucleic acid encoding an MSPI

is used. For example, a retroviral vector can be used (see Miller et al., 1993, Meth. Enzymol. 217:581-599). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA. The nucleic acid encoding the MSPI to be used in gene therapy is cloned into the vector, which facilitates delivery of the gene into a subject. More detail about retroviral vectors can be found in Boesen et al., 1994, Biotherapy 6:291-302, which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., 1994, J. Clin. Invest. 93:644-651; Kiem et al., 1994, Blood 83:1467-1473; Salmons and Gunzberg, 1993, Human Gene Therapy 4:129-141; and Grossman and Wilson, 1993, Curr. Opin. in Genetics and Devel. 3:110-114.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, Current Opinion in Genetics and Development 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, Human Gene Therapy 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, Science 252:431-434; Rosenfeld et al., 1992, Cell 68:143-155; Mastrangeli et al., 1993, J. Clin. Invest. 91:225-234; WO94/12649; and Wang, et al., 1995, Gene Therapy 2:775-783.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, Proc. Soc. Exp. Biol. Med. 204:289-300; U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a subject.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, 1993, Meth. Enzymol. 217:599-618; Cohen et al., 1993, Meth. Enzymol. 217:618-644; Cline, 1985, Pharmac. Ther. 29:69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a subject by various methods known in the art. In a preferred embodiment, epithelial cells are injected, e.g., subcutaneously.

In another embodiment, recombinant skin cells may be applied as a skin graft onto the subject. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, the condition of the subject, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to neuronal cells, glial cells (e.g., oligodendrocytes or astrocytes), epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood or fetal liver.

In a preferred embodiment, the cell used for gene therapy is autologous to the subject that is treated.

In an embodiment in which recombinant cells are used in gene therapy, a nucleic acid encoding an MSPI is introduced into the cells such that it is expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem or progenitor cells which can be isolated and maintained in vitro can be used in accordance with this embodiment of the present invention (see e.g. WO 94/08598; Stemple and Anderson, 1992; Cell 71:973-985; Rheinwald, 1980, Meth. Cell Bio. 21A:229; and Pittelkow and Scott, 1986, Mayo Clinic Proc. 61:771).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

Direct injection of a DNA coding for an MSPI may also be performed according to, for example, the techniques described in United States Patent No. 5,589,466. These techniques involve the injection of "naked DNA", i.e., isolated DNA molecules in the absence of liposomes, cells, or any other material besides a suitable carrier. The injection of DNA encoding a protein and operably linked to a suitable promoter results in the production of the protein in cells near the site of injection and the elicitation of an immune response in the subject to the protein encoded by the injected DNA. In a preferred embodiment, naked DNA comprising (a) DNA encoding an MSPI and (b) a promoter are injected into a subject to elicit an immune response to the MSPI.

5.16.3 Inhibition of MSPIs to Treat MS

In one embodiment of the invention, MS is treated or prevented by administration of a agent that antagonizes (inhibits) the level(s) and/or function(s) of one or more MSPIs which are elevated in a body fluid sample of subjects having MS as compared with a body fluid sample of subjects free from MS. Agents useful for this purpose include but are not limited to anti-MSPI antibodies (and fragments and derivatives containing the binding region thereof), MSPI antisense or ribozyme nucleic acids, and nucleic acids encoding dysfunctional MSPIs that are used to "knockout" endogenous MSPI function by homologous recombination (see, e.g., Capecchi, 1989, Science 244:1288-1292). Other agents that inhibit MSPI function can be

identified by use of known in vitro assays, e.g., assays for the ability of a test agent to inhibit binding of an MSPI to another protein or a binding partner, or to inhibit a known MSPI function. Preferably such inhibition is assayed in vitro or in cell culture, but genetic assays may also be employed. The Preferred Technology can also be used to detect levels of the MSPIs before and after the administration of the agent. Preferably, suitable in vitro or in vivo assays are utilized to determine the effect of a specific agent and whether its administration is indicated for treatment of the affected tissue, as described in more detail below.

In a specific embodiment, a agent that inhibits an MSPI function is administered therapeutically or prophylactically to a subject in whom an increased CSF level or functional activity of the MSPI (e.g., greater than the normal level or desired level) is detected as compared with CSF of subjects free from MS or a predetermined reference range. Methods standard in the art can be employed to measure the increase in an MSPI level or function, as outlined above. Preferred MSPI inhibitor compositions include small molecules, i.e., molecules of 1000 daltons or less. Such small molecules can be identified by the screening methods described herein.

In a further embodiment, an MSPI may be seen to be decreased in the CSF where this decrease represents an increase in the MSPI level in another compartment, for example but without limitation sequestering of the MSPI in a cell, subcellular compartment, body fluid or tissue rather than secretion into CSF. Under these conditions, a agent that inhibits an MSPI function is administered therapeutically or prophylactically to a subject in whom a decreased CSF level or functional activity of the MSPI (e.g., greater than the normal level or desired level) is detected as compared with CSF of subjects free from MS or a predetermined reference range.

5.16.4 Antisense Regulation of MSPIs

In a specific embodiment, MSPI expression is inhibited by use of MSPI antisense nucleic acids. The present invention provides the therapeutic or prophylactic use of nucleic acids comprising at least six nucleotides that are antisense to a gene or cDNA encoding an MSPI or a portion thereof. As used herein, an MSPI "antisense" nucleic acid refers to a nucleic acid capable of hybridizing by virtue of some sequence complementarity to a portion of an RNA (preferably mRNA) encoding an MSPI. The antisense nucleic acid may be complementary to a coding and/or noncoding region of an mRNA encoding an MSPI. Such antisense nucleic acids have utility as agents that inhibit MSPI expression, and can be used in the treatment or prevention of MS.

The antisense nucleic acids of the invention are double-stranded or single-stranded oligonucleotides, RNA or DNA or a modification or derivative thereof, and can be directly administered to a cell or produced intracellularly by transcription of exogenous, introduced sequences.

The invention further provides pharmaceutical compositions comprising an effective amount of the MSPI antisense nucleic acids of the invention in a pharmaceutically acceptable carrier, as described infra.

In another embodiment, the invention provides methods for inhibiting the expression of an MSPI nucleic acid sequence in a prokaryotic or eukaryotic cell comprising providing the cell with an effective amount of a composition comprising an MSPI antisense nucleic acid of

the invention.

MSPI antisense nucleic acids and their uses are described in detail below.

5.16.5 MSPI Antisense Nucleic Acids

The MSPI antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides ranging from 6 to about 50 oligonucleotides. In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof and can be single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appended groups such as peptides; agents that facilitate transport across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. USA 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; WO 88/09810,) or blood-brain barrier (see, e.g., WO 89/10134,); hybridization-triggered cleavage agents (see, e.g., Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents (see, e.g., Zon, 1988, Pharm. Res. 5:539-549).

In a preferred aspect of the invention, an MSPI antisense oligonucleotide is provided, preferably of single-stranded DNA. The oligonucleotide may be modified at any position on its structure with substituents generally known in the art.

The MSPI antisense oligonucleotide may comprise at least one of the following modified base moieties: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5 - methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, 2,6-diaminopurine, and other base analogs.

In another embodiment, the oligonucleotide comprises at least one modified sugar moiety, e.g., one of the following sugar moieties: arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the oligonucleotide comprises at least one of the following modified phosphate backbones: a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, a formacetal, or an analog of formacetal.

In yet another embodiment, the oligonucleotide is an alpha-anomeric oligonucleotide. An alpha-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual alpha -units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641).

The oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage

agent.

Oligonucleotides of the invention may be synthesized by standard methods known in the art, e.g., by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), and methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. USA 85:7448-7451).

In a specific embodiment, the MSPI antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced in vivo such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the MSPI antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the MSPI antisense RNA can be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible or constitutive. Examples of such promoters are outlined above.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene encoding an MSPI, preferably a human gene encoding an MSPI. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridize under stringent conditions (e.g., highly stringent conditions or moderately stringent conditions as defined *supra*) with the RNA, forming a stable duplex; in the case of double-stranded MSPI antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA encoding an MSPI it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

5.16.6 Therapeutic Use of MSPI Antisense Nucleic Acids

The MSPI antisense nucleic acids can be used to treat or prevent MS when the target MSPI is overexpressed in the CSF of subjects suspected of having or suffering from MS. In a preferred embodiment; a single-stranded DNA antisense MSPI oligonucleotide is used.

Cell types which express or overexpress RNA encoding an MSPI can be identified by various methods known in the art. Such cell types include but are not limited to leukocytes (e.g., neutrophils, macrophages, monocytes) and resident cells (e.g., astrocytes, glial cells, neuronal cells, and ependymal cells). Such methods include, but are not limited to, hybridization with an MSPI-specific nucleic acid (e.g., by Northern hybridization, dot blot hybridization, in situ hybridization), observing the ability of RNA from the cell type to be translated in vitro into an MSPI, immunoassay, etc. In a preferred aspect, primary tissue from

a subject can be assayed for MSPI expression prior to treatment, e.g., by immunocytochemistry or in situ hybridization.

Pharmaceutical compositions of the invention, comprising an effective amount of an MSPI antisense nucleic acid in a pharmaceutically acceptable carrier, can be administered to a subject having MS.

The amount of MSPI antisense nucleic acid which will be effective in the treatment of MS can be determined by standard clinical techniques.

In a specific embodiment, pharmaceutical compositions comprising one or more MSPI antisense nucleic acids are administered via liposomes, microparticles, or microcapsules. In various embodiments of the invention, such compositions may be used to achieve sustained release of the MSPI antisense nucleic acids.

5.16.7 Inhibitory Ribozyme andTriple Helix Approaches

In another embodiment, symptoms of MS may be ameliorated by decreasing the level of an MSPI or MSPI activity by using gene sequences encoding the MSPI in conjunction with well-known gene "knock-out," ribozyme or triple helix methods to decrease gene expression of an MSPI. In this approach ribozyme or triple helix molecules are used to modulate the activity, expression or synthesis of the gene encoding the MSPI, and thus to ameliorate the symptoms of MS. Such molecules may be designed to reduce or inhibit expression of a mutant or non-mutant target gene. Techniques for the production and use of such molecules are well known to those of skill in the art.

Ribozyme molecules designed to catalytically cleave gene mRNA transcripts encoding an MSPI can be used to prevent translation of target gene mRNA and, therefore, expression of the gene product. (See, e.g., WO90/11364; Sarver et al., 1990, Science 247:1222-1225).

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. (For a review, see Rossi, 1994, Current Biology 4, 469-471). The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, e.g., U.S. Patent No. 5,093,246, which is incorporated herein by reference in its entirety.

While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs encoding an MSPI, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Myers, 1995, Molecular Biology and Biotechnology: A Comprehensive Desk Reference, VCH Publishers, New York, (see especially Figure 4, page 833) and in Haseloff and Gerlach, 1988, Nature, 334, 585-591, each of which is incorporated herein by reference in its entirety.

Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA encoding the MSPI, i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one that occurs naturally in Tetrahymena thermophila (known as the IVS, or L-19 IVS RNA) and that has been extensively described by Thomas Cech and collaborators (Zaug, et al., 1984, Science, 224, 574-578; Zaug and Cech, 1986, Science, 231, 470-475; Zaug, et al., 1986, Nature, 324, 429-433; published International patent application No. WO 88/04300 by University Patents Inc.; Been and Cech, 1986, Cell, 47, 207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes which target eight base-pair active site sequences that are present in the gene encoding the MSPI.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.) and should be delivered to cells that express the MSPI in vivo. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous mRNA encoding the MSPI and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficacy.

Endogenous MSPI expression can also be reduced by inactivating or "knocking out" the gene encoding the MSPI, or the promoter of such a gene, using targeted homologous recombination (e.g., see Smithies, et al., 1985, Nature 317:230-234; Thomas and Capecchi, 1987, Cell 51:503-512; Thompson et al., 1989, Cell 5:313-321; and Zijlstra et al., 1989, Nature 342:435-438, each of which is incorporated by reference herein in its entirety). For example, a mutant gene encoding a non-functional MSPI (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous gene (either the coding regions or regulatory regions of the gene encoding the MSPI) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express the target gene in vivo. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the target gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive target gene (e.g., see Thomas and Capecchi, 1987 and Thompson, 1989, supra). However this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site in vivo using appropriate viral vectors.

Alternatively, the endogenous expression of a gene encoding an MSPI can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the gene (i.e., the gene promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene encoding the MSPI in target cells in the body. (See generally, Helene, 1991, Anticancer Drug Des., 6(6), 569-584; Helene, et al., 1992, Ann. N.Y. Acad. Sci., 660, 27-36; and Maher, 1992, Bioassays 14(12), 807-815).

Nucleic acid molecules to be used in triplex helix formation for the inhibition of transcription should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleotide sequences may be

pyrimidine-based, which will result in TAT and CGC+ triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich molecules provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules may be chosen that are purine-rich, for example, contain a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

In instances wherein the antisense, ribozyme, or triple helix molecules described herein are utilized to inhibit mutant gene expression, it is possible that the technique may so efficiently reduce or inhibit the transcription (triple helix) or translation (antisense, ribozyme) of mRNA produced by normal gene alleles of an MSPI that the situation may arise wherein the concentration of MSPI present may be lower than is necessary for a normal phenotype. In such cases, to ensure that substantially normal levels of activity of a gene encoding an MSPI are maintained, gene therapy may be used to introduce into cells nucleic acid molecules that encode and express the MSPI that exhibit normal gene activity and that do not contain sequences susceptible to whatever antisense, ribozyme, or triple helix treatments are being utilized. Alternatively, in instances whereby the gene encodes an extracellular protein, normal MSPIs can be co-administered in order to maintain the requisite level of MSPI activity.

Antisense RNA and DNA, ribozyme, and triple helix molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules, as discussed above. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

5.17 ASSAYS FOR THERAPEUTIC OR PROPHYLACTIC AGENTS

The present invention also provides assays for use in drug discovery in order to identify or verify the efficacy of agents for treatment or prevention of MS. Candidate agents can be assayed for their ability to restore MSF or MSPI levels in a subject having MS towards levels found in subjects free from MS or to produce similar changes in experimental animal models of MS. Agents able to restore MSF or MSPI levels in a subject having MS towards levels found in subjects free from MS or to produce similar changes in experimental animal models of MS can be used as lead agents for further drug discovery, or used therapeutically. MSF and MSPI expression can be assayed by the Preferred Technology, immunoassays, gel

electrophoresis followed by visualization, detection of MSPI activity, or any other method taught herein or known to those skilled in the art. Such assays can be used to screen candidate drugs, in clinical monitoring or in drug development, where abundance of an MSF or MSPI can serve as a surrogate marker for clinical disease.

In various specific embodiments, in vitro assays can be carried out with cells representative of cell types involved in a subject's disorder, to determine if a agent has a desired effect upon such cell types.

Agents for use in therapy can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chickens, cows, monkeys, rabbits, etc. For in vivo testing, prior to administration to humans, any animal model system known in the art may be used Examples of animal models of MS include, but are not limited to, experimental autoimmune encephalomyelitis (EAE) (Steinman (1999) Neuron, 24:511-514). It is also apparent to the skilled artisan that, based upon the present disclosure, transgenic animals can be produced with "knock-out" mutations of the gene or genes encoding one or more MSPIs. A "knock-out" mutation of a gene is a mutation that causes the mutated gene to not be expressed, or expressed in an aberrant form or at a low level, such that the activity associated with the gene product is nearly or entirely absent. Preferably, the transgenic animal is a mammal, more preferably, the transgenic animal is a mouse.

In one embodiment, candidate agents that modulate the expression of an MSPI are identified in non-human animals (e.g., mice, rats, monkeys, rabbits, and guinea pigs), preferably non-human animal models for MS, expressing the MSPI. In accordance with this embodiment, a candidate agent or a control agent is administered to the animals, and the effect of the candidate agent on expression of one or more MSPIs is determined. A candidate agent that alters the expression of an MSPI (or a plurality of MSPIs) can be identified by comparing the level of the selected MSPI or MSPIs (or mRNA(s) encoding the same) in an animal or group of animals treated with a candidate agent with the level of the MSPIs(s) or mRNA(s) in an animal or group of animals treated with a control agent. Techniques known to those of skill in the art can be used to determine the mRNA and protein levels, for example, in situ hybridization. The animals may or may not be sacrificed to assay the effects of a candidate agent.

In another embodiment, candidate agents that modulate the activity of an MSPI or a biologically active portion thereof are identified in non-human animals (e.g., mice, rats, monkeys, rabbits, and guinea pigs), preferably non-human animal models for MS, expressing the MSPIs. In accordance with this embodiment, a candidate agent or a control agent is administered to the animals, and the effect of a candidate agent on the activity of an MSPI is determined. A candidate agent that alters the activity of an MSPI (or a plurality of MSPIs) can be identified by assaying animals treated with a control agent and animals treated with the candidate agent. The activity of the MSPI can be assessed by detecting induction of a cellular second messenger of the MSPI (e.g., intracellular $Ca2^+$, diacylglycerol, IP3, etc.), detecting catalytic or enzymatic activity of the MSPI or binding partner thereof, detecting the induction of a reporter gene (e.g., a regulatory element that is responsive to an MSPI of the invention operably linked to a nucleic acid encoding a detectable marker, such as luciferase or green fluorescent protein), or detecting a cellular response (e.g., cellular differentiation or cell proliferation). Techniques known to those of skill in the art can be utilized to detect changes

in the activity of an MSPI (see, e.g., U.S.. 5,401,639,).

In yet another embodiment, candidate agent that modulate the level or expression of an MSPI (or plurality of MSPIs) are identified in human subjects having MS, preferably those having severe MS. In accordance with this embodiment, a candidate agent or a control agent is administered to the human subject, and the effect of a candidate agent on MSPI expression is determined by analyzing the expression of the MSPI or the mRNA encoding the same in a biological sample (e.g., CSF, serum, plasma, or urine). A candidate agent that alters the expression of an MSPI can be identified by comparing the level of the MSPI or mRNA encoding the same in a subject or group of subjects treated with a control agent to that in a subject or group of subjects treated with a candidate agent. Alternatively, alterations in the expression of an MSPI can be identified by comparing the level of the MSPI or mRNA encoding the same in a subject or group of subjects before and after the administration of a candidate agent. Techniques known to those of skill in the art can be used to obtain the biological sample and analyze the mRNA or protein expression. For example, the Preferred Technology described herein can be used to assess changes in the level of an MSPI.

In another embodiment, candidate agents that modulate the activity of an MSPI (or plurality of MSPIs) are identified in human subjects having MS, preferably those having severe MS. In this embodiment, a candidate agent or a control agent is administered to the human subject, and the effect of a candidate agent on the activity of an MSPI is determined. A test com candidate agent pound that alters the activity of an MSPI can be identified by comparing biological samples from subjects treated with a control agent to samples from subjects treated with the candidate agent. Alternatively, alterations in the activity of an MSPI can be identified by comparing the activity of an MSPI in a subject or group of subjects before and after the administration of a candidate agent. The activity of the MSPI can be assessed by detecting in a biological sample (e.g., CSF, serum, plasma, or urine) induction of a cellular signal transduction pathway of the MSPI (e.g., intracellular $Ca2^{+}$, diacylglycerol, IP3, etc.), catalytic or enzymatic activity of the MSPI or a binding partner thereof, or a cellular response, for example, cellular differentiation, or cell proliferation. Techniques known to those of skill in the art can be used to detect changes in the induction of a downstream effector of an MSPI or changes in a cellular response. For example, RT-PCR can be used to detect changes in the induction of a downstream effector.

In a preferred embodiment, a candidate agent that changes the level or expression of an MSPI towards levels detected in control subjects (e.g., humans free from MS) is selected for further testing or therapeutic use. In another preferred embodiment, a candidate agent that changes the activity of an MSPI towards the activity found in control subjects (e.g., humans free from MS) is selected for further testing or therapeutic use.

In another embodiment, candidate agent that reduce the severity of one or more symptoms associated with MS are identified in human subjects having MS, preferably subjects severe MS. In accordance with this embodiment, a candidate agent or a control agent is administered to the subjects, and the effect of a candidate agent on one or more symptoms of MS is determined. A candidate agent that reduces one or more symptoms can be identified by comparing the subjects treated with a control agent to the subjects treated with the candidate agent. Techniques known to physicians familiar with MS can be used to determine whether a candidate agent reduces one or more symptoms associated with MS. For example, a candidate

agent that reduces clinical relapse rate, development of new MRI lesions, delays disability progression, enhances speed of clinical recovery, restores blood brain barrier status on MRI or delays increase of MRI lesion volume, protection of oligodendrocytes, axonal remyelination in a subject having MS will be beneficial for treating subjects having MS.

In a preferred embodiment, a candidate agent that reduces the severity of one or more symptoms associated with MS in a human having MS is selected for further testing or therapeutic use.

5.18 THERAPEUTIC AND PROPHYLACTIC COMPOSITIONS AND THEIR USE

The invention provides methods of treatment (and prophylaxis) comprising administering to a subject an effective amount of an active agent. An "active agent" as used herein comprises MSPIs, MSPI fragments, MSPI-related polypeptides, anti-MSPI antibodies, fragments of anti-MSPI antibodes and agents which modulate the expression of MSPIs e.g. agonists and antagonists of MSPIs. In a preferred aspect, the agent is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the agent comprises a nucleic acid are described above; additional appropriate formulations and routes of administration are described below. A "pharmaceutical composition" as used herein comprises an active agent optionally with a pharmaceutically acceptable carrier.

Various delivery systems are known and can be used to administer a agent of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the agent, receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction can be enteral or parenteral and include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The agents may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, e.g., by injection, by means of a catheter, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection into CSF or at the site (or former site) of neurodegeneration or to CNS tissue.

In another embodiment, the agent can be delivered in a vesicle, in particular a liposome (see Langer, 1990, Science 249:1527-1533; Treat et al., in Liposomes in the

Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)

In yet another embodiment, the agent can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., 1983, Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 71:105). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, e.g. the CNS, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533).

In a specific embodiment where the agent of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo as described supra.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a agent, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences", Ed. E.W. Martin, ISBN: 0-912734-04-3, Mack Publishing Co. Such compositions will contain a therapeutically effective amount of the agent, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject. The formulation should suit the mode of administration.

In a specific embodiment, the composition is formulated in accordance with routine

procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The agents of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the agent of the invention which will be effective in the treatment of MS can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the active agent, the route of administration of the active agent, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active agent per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects (a) approval by the agency of manufacture, use or sale for human administration, (b) directions for use, or both.

6. EXAMPLE: IDENTIFICATION OF PROTEINS DIFFERENTIALLY EXPRESSED IN THE CSF IN MS

Using the following procedure, proteins in CSF samples from 7 subjects having MS and 7 control subjects were separated by isoelectric focusing followed by SDS-PAGE and analyzed. Parts 6.1.1 to 6.1.14 (inclusive) of the procedure set forth are hereby designated as the "Reference Protocol"

6.1 MATERIALS AND METHODS

6.1.1 Sample Preparation

A protein assay (Pierce BCA Cat # 23225) was performed on each CSF sample as

received. Prior to protein separation, each sample was processed for selective depletion of certain proteins, in order to enhance and simplify protein separation and facilitate analysis by removing proteins that may interfere with or limit analysis of proteins of interest. See WO 99/63351, which is incorporated by reference in its entirety, with particular reference to pages 3 and 6.

Removal of albumin, haptoglobin, transferrin and immunoglobin G (IgG) from CSF ("CSF depletion") was achieved by an affinity chromatography purification step in which the sample was passed through a series of Hi-Trap™ columns containing immobilized antibodies for selective removal of albumin, haptoglobin and transferrin, and protein G for selective removal of immunoglobin G. Two affinity columns in a tandem assembly were prepared by coupling antibodies to protein G-sepharose contained in Hi-Trap™ columns (Protein G-Sepharose Hi-Trap columns (1 ml) Pharmacia Cat. No. 17-0404-01). This was done by circulating the following solutions sequentially through the columns: (1) Dulbecco's Phosphate Buffered Saline (Gibco BRL Cat. No. 14190-094); (2) concentrated antibody solution; (3) 200 mM sodium carbonate buffer, pH 8.35; (4) cross-linking solution (200 mM sodium carbonate buffer, pH 8.35, 20 mM dimethylpimelimidate); and (5) 500 mM ethanolamine, 500 mM NaCl. A third (un-derivatised) protein G Hi-Trap column was then attached to the lower end of the tandem column assembly. .

The chromatographic procedure was automated using an Akta Fast Protein Liquid Chromatography (FPLC) System such that a series of up to seven runs could be performed sequentially. The samples were passed through the series of 3 Hi-Trap columns in which the affinity chromatography media selectively bind the above proteins thereby removing them from the sample. Fractions (typically 3 ml per tube) were collected of unbound material ("Flowthrough fractions") that eluted through the column during column loading and washing stages and of bound proteins ("Bound/Eluted fractions") that were eluted by step elution with Immunopure Gentle Ag/Ab Elution Buffer (Pierce Cat. No. 21013). The eluate containing unbound material was collected in fractions which were pooled, desalted/concentrated by centrifugal ultrafiltration and stored to await further analysis by 2D PAGE.

A volume of depleted CSF containing approximately 100-150 µg of total protein was aliquoted and an equal volume of 10% (w/v) SDS (Fluka 71729), 2.3% (w/v) dithiothreitol (BDH 443852A) was added. The sample was heated at 95°C for 5 mins, and then allowed to cool to 20°C. 125µl of the following buffer was then added to the sample:

8M urea (BDH 452043w)
4% CHAPS (Sigma C3023)
65mM dithiotheitol (DTT)
2% (v/v) Resolytes™ 3.5-10 (BDH 44338 2x)

This mixture was vortexed, and centrifuged at 13000 rpm for 5 mins at 15°C, and the supernatant was analyzed by isoelectric focusing.

6.1.2 Isoelectric Focusing

Isoelectric focusing (IEF), was performed using the Immobiline™ DryStrip Kit (Pharmacia BioTech), following the procedure described in the manufacturer's instructions, see Instructions for Immobiline™ DryStrip Kit, Pharmacia, # 18-1038-63, Edition AB

(incorporated herein by reference in its entirety). Immobilized pH Gradient (IPG) strips (18cm, pH 3-10 non-linear strips; Pharmacia Cat. # 17-1235-01) were rehydrated overnight at 20°C in a solution of 8M urea, 2% (w/v) CHAPS, 10mM DTT, 2% (v/v) Resolytes 3.5-10, as described in the Immobiline DryStrip Users Manual. For IEF, 50ml of supernatant (prepared as above) was loaded onto a strip, with the cup-loading units being placed at the basic end of the strip. The loaded gels were then covered with mineral oil (Pharmacia 17-3335-01) and a voltage was immediately applied to the strips according to the following profile, using a Pharmacia EPS3500XL power supply (Cat 19-3500-01):

Initial voltage = 300V for 2 hrs
Linear Ramp from 300V to 3500V over 3hrs
Hold at 3500V for 19hrs

For all stages of the process, the current limit was set to 10mA for 12 gels, and the wattage limit to 5W. The temperature was held at 20°C throughout the run.

6.1.3 Gel Equilibration and SDS-PAGE

After the final 19hr step, the strips were immediately removed and immersed for 10 mins at 20°C in a first solution of the following composition: 6M urea; 2% (w/v) DTT; 2% (w/v) SDS; 30% (v/v) glycerol (Fluka 49767); 0.05M Tris/HCl, pH 6.8 (Sigma Cat T-1503). The strips were removed from the first solution and immersed for 10 mins at 20°C in a second solution of the following composition: 6M urea; 2% (w/v) iodoacetamide (Sigma I-6125); 2% (w/v) SDS; 30% (v/v) glycerol; 0.05M Tris/HCl, pH 6.8. After removal from the second solution, the strips were loaded onto supported gels for SDS-PAGE according to Hochstrasser et al., 1988, Analytical Biochemistry 173: 412-423 (incorporated herein by reference in its entirety), with modifications as specified below.

6.1.4 Preparation of supported gels

The gels were cast between two glass plates of the following dimensions: 23cm wide x 24cm long (back plate); 23cm wide x 24cm long with a 2cm deep notch in the central 19cm (front plate). To promote covalent attachment of SDS-PAGE gels, the back plate was treated with a 0.4% solution of g-methacryl-oxypropyltrimethoxysilane in ethanol (BindSilaneJ; Pharmacia Cat. # 17-1330-01). The front plate was treated with a 2% solution of dimethyldichlorosilane dissolved in octamethyl cyclo-octasilane (RepelSilaneJ Pharmacia Cat. # 17-1332-01) to reduce adhesion of the gel. Excess reagent was removed by washing with water, and the plates were allowed to dry. At this stage, both as identification for the gel, and as a marker to identify the coated face of the plate, an adhesive bar-code was attached to the back plate in a position such that it would not come into contact with the gel matrix.

The dried plates were assembled into a casting box with a capacity of 13 gel sandwiches. The top and bottom plates of each sandwich were spaced by means of 1mm thick spacers, 2.5 cm wide. The sandwiches were interleaved with acetate sheets to facilitate separation of the sandwiches after gel polymerization. Casting was then carried out according to Hochstrasser et al., op. cit.

A 9-16% linear polyacrylamide gradient was cast, extending up to a point 2cm below

the level of the notch in the front plate, using the Angelique gradient casting system (Large Scale Biology). Stock solutions were as follows. Acrylamide (40% in water) was from Serva (Cat. # 10677). The cross-linking agent was PDA (BioRad 161-0202), at a concentration of 2.6% (w/w) of the total starting monomer content. The gel buffer was 0.375M Tris/HCl, pH 8.8. The polymerization catalyst was 0.05% (v/v) TEMED (BioRad 161-0801), and the initiator was 0.1% (w/v) APS (BioRad 161-0700). No SDS was included in the gel and no stacking gel was used. The cast gels were allowed to polymerize at 20°C overnight, and then stored at 4°C in sealed polyethylene bags with 6ml of gel buffer, and were used within 4 weeks.

6.1.5 SDS-PAGE

A solution of 0.5% (w/v) agarose (Fluka Cat 05075) was prepared in running buffer (0.025M Tris, 0.198M glycine (Fluka 50050), 1% (w/v) SDS, supplemented by a trace of bromophenol blue). The agarose suspension was heated to 70°C with stirring, until the agarose had dissolved. The top of the supported 2nd D gel was filled with the agarose solution, and the equilibrated strip was placed into the agarose, and tapped gently with a palette knife until the gel was intimately in contact with the 2nd D gel. The gels were placed in the 2nd D running tank, as described by Amess et al., 1995, Electrophoresis 16: 1255-1267 (incorporated herein by reference in its entirety). The tank was filled with running buffer (as above) until the level of the buffer was just higher than the top of the region of the 2nd D gels which contained polyacrylamide, so as to achieve efficient cooling of the active gel area. Running buffer was added to the top buffer compartments formed by the gels, and then voltage was applied immediately to the gels using a Consort E-833 power supply. For 1 hour, the gels were run at 20mA/gel. The wattage limit was set to 150W for a tank containing 6 gels, and the voltage limit was set to 600V. After 1 hour, the gels were then run at 40mA/gel, with the same voltage and wattage limits as before, until the bromophenol blue line was 0.5cm from the bottom of the gel. The temperature of the buffer was held at 16°C throughout the run. Gels were not run in duplicate.

6.1.6 Staining

Upon completion of the electrophoresis run, the gels were immediately removed from the tank for fixation. The top plate of the gel cassette was carefully removed, leaving the gel bonded to the bottom plate. The bottom plate with its attached gel was then placed into a staining apparatus, which can accommodate 12 gels. The gels were completely immersed in fixative solution of 40% (v/v) ethanol (BDH 28719), 10% (v/v) acetic acid (BDH 100016X), 50% (v/v) water (MilliQ-Millipore), which was continuously circulated over the gels. After an overnight incubation, the fixative was drained from the tank, and the gels were primed by immersion in 7.5% (v/v) acetic acid, 0.05% (w/v) SDS, 92.5% (v/v) water for 30 mins. The priming solution was then drained, and the gels were stained by complete immersion for 4 hours in a staining solution of Pyridinium, 4-[2-[4-(dipentylamino)-2-trifluoromethylphenyl] ethenyl]-1-(sulfobutyl)-, inner salt, prepared by diluting a stock solution of this dye (2mg/ml in DMSO) in 7.5% (v/v) aqueous acetic acid to give a final concentration of 1.2 mg/l; the staining solution was vacuum filtered through a 0.4μm filter (Duropore) before use.

6.1.7 Imaging of the gel

A computer-readable output was produced by imaging the fluorescently stained gels with the preferred scanner (Oxford Glycosciences, Oxford, UK described in section 5.2, supra. This scanner has a gel carrier with four integral fluorescent markers (Designated M1, M2, M3, M4) that are used to correct the image geometry and are a quality control feature to confirm that the scanning has been performed correctly.

For scanning, the gels were removed from the stain, rinsed with water and allowed to air dry briefly, and imaged on the preferred scanner. After imaging, the gels were sealed in polyethylene bags containing a small volume of staining solution, and then stored at 4°C.

6.1.8 Digital Analysis of the Data

The data were processed as described in WO 98/23950 at Sections 5.4 and 5.5 (incorporated herein by reference), as set forth more particularly below.

The output from the scanner was first processed using the MELANIE7 II 2D PAGE analysis program (Release 2.2, 1997, BioRad Laboratories, Hercules, California, Cat. # 170-7566) to autodetect the registration points, M1, M2, M3 and M4; to autocrop the images (i.e., to eliminate signals originating from areas of the scanned image lying outside the boundaries of the gel, e.g. the reference frame); to filter out artifacts due to dust; to detect and quantify features; and to create image files in GIF format. Features were detected using the following parameters:

Smooths =2
Laplacian threshold 50
Partials threshold.1
Saturation = 100
Peakedness = 0
Minimum Perimeter = 10

6.1.9 Assignment of pI and MW Values

Landmark identification was used to determine the pI and MW of features detected in the images. Twelve landmark features, designated CSF1 to CSF12, were identified in a standard CSF image obtained from a pooled sample. These landmark features are identified in Figure 1 and were assigned the pI and/or MW values identified in Table X.

Table X. Landmark Features Used In This Study

Name	pI	MW (Da)	Name	pI	MW (Da)
CSF1	5.96	185230	CSF7	4.78	41340
CSF2	5.39	141700	CSF8	9.2	40000
CSF3	6.29	100730	CSF9	5.5	31900
CSF4	5.06	71270	CSF10	6.94	27440
CSF5	7.68	68370	CSF11	5.9	23990
CSF6	5.67	48090	CSF12	6.43	10960

As many of these landmarks as possible were identified in each gel image of the dataset. Each feature in the study gels was then assigned a pI value by linear interpolation or extrapolation (using the MELANIE7-II software) to the two nearest landmarks, and was assigned a MW value by linear interpolation or extrapolation (using the MELANIE7-II software) to the two nearest landmarks.

6.1.10 Matching With Primary Master Image

Images were edited to remove gross artifacts such as dust, to reject images which had gross abnormalities such as smearing of protein features, or were of too low a loading or overall image intensity to allow identification of more than the most intense features, or were of too poor a resolution to allow accurate detection of features. Images were then compared by pairing with one common image from the whole sample set. This common image, the "primary master image", was selected on the basis of protein load (maximum load consistent with maximum feature detection), a well resolved myoglobin region, (myoglobin was used as an internal standard), and general image quality. Additionally, the primary master image was chosen to be an image which appeared to be generally representative of all those to be included in the analysis. (This process by which a primary master gel was judged to be representative of the study gels was rechecked by the method described below and in the event that the primary master gel was seen to be unrepresentative, it was rejected and the process repeated until a representative primary master gel was found.)

Each of the remaining study gel images was individually matched to the primary master image such that common protein features were paired between the primary master image and each individual study gel image as described below.

6.1.11 Cross-matching Between Samples

To facilitate statistical analysis of large numbers of samples for purposes of identifying features that are differentially expressed, the geometry of each study gel was adjusted for maximum alignment between its pattern of protein features, and that of the primary master, as follows. Each of the study gel images was individually transformed into the geometry of the primary master image using a multi-resolution warping procedure. This procedure corrects the image geometry for the distortions brought about by small changes in the physical parameters of the electrophoresis separation process from one sample to another. The observed changes are such that the distortions found are not simple geometric distortions, but rather a smooth

flow, with variations at both local and global scale.

The fundamental principle in multi-resolution modeling is that smooth signals may be modeled as an evolution through 'scale space', in which details at successively finer scales are added to a low resolution approximation to obtain the high resolution signal. This type of model is applied to the flow field of vectors (defined at each pixel position on the reference image) and allows flows of arbitrary smoothness to be modeled with relatively few degrees of freedom. Each image is first reduced to a stack, or pyramid, of images derived from the initial image, but smoothed and reduced in resolution by a factor of 2 in each direction at every level (Gaussian pyramid) and a corresponding difference image is also computed at each level, representing the difference between the smoothed image and its progenitor (Laplacian pyramid). Thus the Laplacian images represent the details in the image at different scales.

To estimate the distortion between any 2 given images, a calculation was performed at level 7 in the pyramid (i.e. after 7 successive reductions in resolution). The Laplacian images were segmented into a grid of 16x16 pixels, with 50% overlap between adjacent grid positions in both directions, and the cross correlation between corresponding grid squares on the reference and the test images was computed. The distortion displacement was then given by the location of the maximum in the correlation matrix. After all displacements had been calculated at a particular level, they were interpolated to the next level in the pyramid, applied to the test image, and then further corrections to the displacements were calculated at the next scale.

The warping process brought about good alignment between the common features in the primary master image, and the images for the other samples. The MELANIE7 II 2D PAGE analysis program was used to calculate and record approximately 500-700 matched feature pairs between the primary master and each of the other images. The accuracy of this program was significantly enhanced by the alignment of the images in the manner described above. To improve accuracy still further, all pairings were finally examined by eye in the MelView interactive editing program and residual recognizably incorrect pairings were removed. Where the number of such recognizably incorrect pairings exceeded the overall reproducibility of the Preferred Technology (as measured by repeat analysis of the same biological sample) the gel selected to be the primary master gel was judged to be insufficiently representative of the study gels to serve as a primary master gel. In that case, the gel chosen as the primary master gel was rejected, and different gel was selected as the primary master gel, and the process was repeated.

All the images were then added together to create a composite master image, and the positions and shapes of all the gel features of all the component images were super-imposed onto this composite master as described below.

Once all the initial pairs had been computed, corrected and saved, a second pass was performed whereby the original (unwarped) images were transformed a second time to the geometry of the primary master, this time using a flow field computed by smooth interpolation of the multiple tie-points defined by the centroids of the paired gel features. A composite master image was thus generated by initialising the primary master image with its feature descriptors. As each image was transformed into the primary master geometry, it was digitally summed pixel by pixel into the composite master image, and the features that had not been paired by the procedure outlined above were likewise added to the composite master image

description, with their centroids adjusted to the master geometry using the flow field correction.

The final stage of processing was applied to the composite master image and its feature descriptors, which now represent all the features from all the images in the study transformed to a common geometry. The features were grouped together into linked sets or "clusters", according to the degree of overlap between them. Each cluster was then given a unique identifying index, the molecular cluster index (MCI).

An MCI identifies a set of matched features on different images. Thus an MCI represents a protein or proteins eluting at equivalent positions in the 2D separation in different samples.

6.1.12 Construction of Profiles

After matching all component gels in the study to the final composite master image, the intensity of each feature was measured and stored. The end result of this analysis was the generation of a digital profile which contained, for each identified feature: 1) a unique identification code relative to corresponding feature within the composite master image (MCI), 2) the x, y coordinates of the features within the gel, 3) the isoelectric point (pI) of the features, 4) the apparent molecular weight (MW) of the features, 5) the signal value, 6) the standard deviation for each of the preceding measurements, and 7) a method of linking the MCI of each feature to the master gel to which this feature was matched. By virtue of a Laboratory Information Management System (LIMS), this MCI profile was traceable to the actual stored gel from which it was generated, so that proteins identified by computer analysis of gel profile databases could be retrieved. The LIMS also permitted the profile to be traced back to an original sample or patient.

6.1.13 Statistical Analysis of the Profiles

The complementary statistical strategies specified below were used in the order in which they are listed to identify MSFs from the MCIs within the mastergroup.

(a) The Wilcoxon Rank-Sum test. This test was performed between the control and the MS samples for each MCI basis. The MCIs which recorded a p-value less than or equal to 0.05 were selected as statistically significant MSFs with 95% selectivity.

(b) A second non-overlapping selection strategy is based on qualitative presence or absence alone. Using this procedure, a percentage feature presence was calculated across the control samples and MS samples for each MCI which was a potential MSF based on such qualitative criteria alone, i.e. presence or absence. The MCIs which recorded a percentage feature presence of 95% or more on MS samples and a percentage feature presence of 15% or less on control samples, were selected as the qualitative differential MSFs with 85% selectivity. A second group of qualitative differential MSFs with 85% selectivity were formed by those MCIs which recorded a percentage feature presence of 95% or more on control samples and a percentage feature presence of 15% or less on MS samples.

(c) A third non-overlapping selection strategy is based on the fold change. A fold change representing the ratio of the average normalized protein abundances of the MSFs within an MCI, was calculated for each MCI between each set of controls and MS samples. A 95% confidence limit for the mean of the fold changes was calculated. The MCIs with fold

changes which fall outside the confidence limit were selected as MSFs which met the criteria of the significant fold change threshold with 95% selectivity. Because the MCI fold changes are based on a 95% confidence limit, it follows that the significant fold change threshold is itself 95%.

Application of these three analysis strategies allowed MSFs to be selected on the basis of: (a) statistical significance as measured by the Wilcoxon Rank-Sum test, (b) qualitative differences with a chosen selectivity, or (c) a significant fold change threshold with a chosen selectivity,

6.1.14 Recovery and analysis of selected proteins

Proteins in MSFs were robotically excised and processed to generate tryptic digest peptides. Tryptic peptides were analyzed by mass spectrometry using a PerSeptive Biosystems Voyager- DETM STR Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectrometer, and selected tryptic peptides were analyzed by tandem mass spectrometry (MS/MS) using a Micromass Quadrupole Time-of-Flight (Q-TOF) mass spectrometer (Micromass, Altrincham, U.K.) equipped with a nanoflowTM electrospray Z-spray source. For partial amino acid sequencing and identification of MSPIs uninterpreted tandem mass spectra of tryptic peptides were searched using the SEQUEST search program (Eng et al., 1994, J. Am. Soc. Mass Spectrom. 5:976-989), version v.C.1. Criteria for database identification included: the cleavage specificity of trypsin; the detection of a suite of a, b and y ions in peptides returned from the database. The database searched was database constructed of protein entries in the non-redundant database held by the National Centre for Biotechnology Information (NCBI) which is accessible at http://www.ncbi.nlm.nih.gov/. Following identification of proteins through spectral-spectral correlation using the SEQUEST program, masses detected in MALDI-TOF mass spectra were assigned to tryptic digest peptides within the proteins identified. In cases where no amino acid sequences could be identified through searching with uninterpreted MS/MS spectra of tryptic digest peptides using the SEQUEST program, tandem mass spectra of the peptides were interpreted manually, using methods known in the art. (In the case of interpretation of low-energy fragmentation mass spectra of peptide ions see Gaskell et al., 1992, Rapid Commun. Mass Spectrom. 6:658-662). The method described in PCT Application No. PCT/GB01/04034, which is incorporated herein by reference in its entirety, was also used to interpret mass spectra.

7. EXAMPLE: DIAGNOSIS AND TREATMENT OF MS

The following example illustrates the use of an MSPI of the invention for screening or diagnosis of MS, determining the prognosis of a MS patient, or monitoring the effectiveness of MS therapy. The following example also illustrates the use of modulators (e.g., agonists or antagonists) of an MSPI of the invention to treat or prevent MS.

Cathepsins are part of the papain superfamily of lysosomal cysteine proteinases that play a major role in intracellular protein degradation (Kirschke, H. and Barrett, A.J. (1987) in Lysosomes: Their role in protein breakdown (Glaumann, H., and Ballard, F.J., eds) 193-238, Academic Press, London). In addition Cathepsin S appears to be essential for antigen presentation by major histocompatibility complex (MHC) class II molecules. Cathepsin may

have putative roles in several pathologies including cancer (Hughes et al. (1998) Proc. Nat. Acad. Sci. 95: 12410-12415) and neuronal cell death (Isahara et. Al (1999) Neuroscience 91:233-249), such as Cathepsin B in post ischaemic hippocampal neuronal death (Yamashima et al. (1998) Eur J Neurosci 5:1723-33). Cathepsin L may participate in various pathological processes including arthritis (Esser et al. (1994) Arthritis Rheum. 37:236-247) and tumor metastasis (Sheahan et al. (1989) Cancer Research 49:3809-3814). Like many other proteases, Cathepsin L is synthesized as an inactive proenzyme, which is subsequently processed to the mature form through cleavage of an amino-terminal proregion, located between the signal sequence and the N terminus of the mature enzyme (Menar et al. (1998) J Biol Chem 273:4478-4484), resulting in isoforms. Thus the proregion may regulate catalytic activity, and has been shown to be a potent inhibitor of the mature enzyme (Carmona et al. (1996) Biochemistry 35:8149-8157).

The expression of Cathepsin L with a molecular weight of 40,820 Da and pI of 4.37 has been shown herein to be significantly increased in the cerebrospinal fluid (CSF) of subjects having MS as compared with the CSF of subjects free from MS (see Table II – MSPI-300). Thus, quantitative detection of Cathepsin L in CSF can be used to diagnose MS, determine the progression of MS or monitor the effectiveness of a therapy for MS.

In one embodiment of the invention, agents that modulate (*e.g.*, downregulate) the expression, activity or both the expression and activity of Cathepsin L are administered to a subject in need of treatment or for prophylaxis of MS. Antibodies that modulate the expression, activity or both the expression and activity of Cathepsin L are suitable for this purpose. In addition, nucleic acids coding for all or a portion of Cathepsin L, or nucleic acids complementary to all or a portion of Cathepsin L, may be administered. Cathepsin L, or fragments of the Cathepsin L polypeptide may also be administered. The invention also provides screening assays to identify additional agents that modulate the expression of Cathepsin L or activity of Cathepsin L. Agents that modulate the expression of Cathepsin L *in vitro* can be identified by comparing the expression of Cathepsin L in cells treated with a candidate agent to the expression of Cathepsin L in cells treated with a control agent (*e.g.*, saline). Methods for detecting expression of Cathepsin L are known in the art and include measuring the level of Cathepsin L RNA (*e.g.*, by northern blot analysis or RT-PCR) and measuring Cathepsin L protein (*e.g.*, by immunoassay or western blot analysis). Agents that modulate the activity of Cathepsin L can be identified by comparing the ability of a candidate agent to agonize or antagonize a function of Cathepsin L, such as its neurotrophic or neuroprotective activity, to the ability of a control agent (*e.g.*, saline) to inhibit the same function of Cathepsin L. Agents capable of modulating Cathepsin L activity are identified as agents suitable for further development as a agent useful for the treatment of MS.

Through the use of such assays, candidate agents may be tested for their ability to antagonize the activity of Cathepsin L.

Agents identified *in vitro* that affect the expression or activity of Cathepsin L can be tested *in vivo* in animal models of MS, or in subjects having MS, to determine their therapeutic efficacy.

When a reference is made herein to a method of treating of preventing a disease or condition using a particular agent or combination of agents, it is to be understood that such a

reference is intended to include the use of that agent or combination of agents in the preparation of a medicament for the treatment of prevention of the disease or condition.

The present invention is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the invention. Functionally equivalent methods and apparatus within the scope of the invention, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications and variations are intended to fall within the scope of the appended claims. The contents of each reference, patent and patent application cited in this application is hereby incorporated by reference in its entirety..

WE CLAIM:

1. A method for screening or diagnosis of Multiple Sclerosis in a subject, for determining the stage or severity of Multiple Sclerosis in a subject, for identifying a subject at risk of developing Multiple Sclerosis, or for monitoring the effect of therapy administered to a subject having Multiple Sclerosis, said method comprising:

(a) analysing a test sample of body fluid from the subject by two-dimensional electrophoresis to generate a two-dimensional array of features, said array comprising one or more of the following Multiple Sclerosis-Associated Features (MSFs): MSF-1, MSF-2, MSF-3, MSF-4, MSF-5, MSF-6, MSF-7, MSF-8, MSF-9, MSF-10, MSF-11, MSF-12, MSF-13, MSF-14, MSF-15, MSF-16, MSF-17, MSF-18, MSF-20, MSF-21, MSF-22, MSF-23, MSF-24, MSF-25, MSF-26, MSF-27, MSF-28, MSF-29, MSF-30, MSF-31, MSF-32, MSF-33, MSF-34, MSF-35, MSF-36, MSF-37, MSF-38, MSF-39, MSF-40, MSF-41, MSF-42, MSF-43, MSF-44, MSF-45, MSF-46, MSF-47, MSF-48, MSF-49, MSF-50, MSF-51, MSF-52, MSF-52, MSF-53, MSF-54, MSF-55, MSF-56, MSF-57, MSF-58, MSF-59, MSF-60, MSF-61, MSF-62, MSF-63, MSF-64, MSF-65, MSF-66, MSF-67, MSF-69, MSF-70, MSF-71, MSF-72, MSF-73, MSF-75, MSF-76, MSF-77, MSF-78, MSF-79, MSF-80, MSF-81, MSF-82, MSF-83, MSF-84, MSF-85, MSF-86, MSF-87, MSF-88, MSF-89, MSF-90, MSF-91, MSF-92, MSF-93, MSF-94, MSF-95, MSF-96, MSF-97, MSF-98, MSF-99, MSF-100, MSF-101, MSF-102, MSF-104, MSF-105, MSF-106, MSF-107, MSF-108, MSF-109, MSF-110, MSF-111, MSF-112, MSF-113, MSF-114, MSF-115, MSF-116, MSF-117, MSF-119, MSF-120, MSF-121, MSF-122, MSF-123, MSF-125, MSF-126, MSF-127, MSF-128, MSF-130, MSF-131, MSF-132, MSF-133, MSF-134, MSF-135, MSF-136, MSF-137, MSF-139, MSF-140, MSF-141, MSF-142, MSF-143, MSF-144, MSF-145, MSF-147, MSF-148, MSF-149, MSF-150, MSF-151, MSF-152, MSF-153, MSF-154, MSF-155, MSF-156, MSF-157, MSF-158, MSF-159, MSF-160, MSF-161, MSF-163, MSF-165, MSF-167, MSF-168, MSF-169, MSF-170, MSF-171, MSF-172, MSF-173, MSF-174, MSF-175, MSF-176, MSF-177, MSF-178, MSF-179, MSF-180, MSF-181, MSF-182, MSF-183, MSF-184, MSF-185, MSF-186, MSF-187, MSF-188, MSF-189, MSF-190, MSF-191, MSF-192, MSF-193, MSF-194, MSF-195, MSF-196, MSF-197, MSF-198, MSF-199, MSF-200, MSF-201, MSF-202, MSF-203, MSF-204, MSF-205, MSF-206, MSF-207, MSF-208, MSF-209, MSF-210, MSF-211, MSF-212, MSF-213, MSF-215, MSF-216, MSF-217, MSF-218, MSF-219, MSF-220, MSF-221, MSF-222, MSF-223, MSF-224, MSF-225, MSF-226, MSF-227, MSF-228, MSF-229, MSF-230, MSF-231, MSF-232, MSF-233, MSF-234, MSF-235, MSF-236, MSF-237, MSF-238, MSF-239, MSF-240, MSF-241, MSF-242, MSF-243, MSF-244, MSF-245, MSF-246, MSF-247, MSF-248, MSF-250, MSF-251, MSF-252, MSF-253, MSF-254, MSF-257, MSF-259, MSF-260, MSF-261, MSF-262, MSF-263, MSF-264, MSF-265, MSF-266, MSF-267, MSF-268, MSF-269, MSF-270, MSF-271, MSF-273, MSF-274, MSF-276, MSF-277, MSF-278, MSF-279, MSF-280, MSF-281, MSF-282, MSF-283, MSF-284, MSF-285, MSF-287, MSF-289, MSF-290, MSF-291, MSF-292, MSF-294, MSF-295, MSF-296, MSF-298, MSF-299, MSF-300, MSF-301, MSF-302, MSF-303, MSF-305, MSF-306, MSF-307, MSF-308, MSF-310, MSF-311, MSF-312, MSF-313, MSF-314, MSF-316, MSF-318, MSF-319,

MSF-320, MSF-321, MSF-322, MSF-323, MSF-324, MSF-325; and

(b) comparing the abundance of the one or more MSFs in the test sample with the abundance of the one or more MSFs in a body fluid sample from one or more subjects free from Multiple Sclerosis, or with a previously determined reference range for that feature in subjects free from Multiple Sclerosis, or with the abundance at least one Expression Reference Feature (ERF) in the test sample.

2. A method for screening or diagnosis of Multiple Sclerosis in a subject, for determining the stage or severity of Multiple Sclerosis in a subject, for identifying a subject at risk of developing Multiple Sclerosis, or for monitoring the effect of therapy administered to a subject having Multiple Sclerosis, said method comprising quantitatively detecting, in a test sample of body fluid from the subject, one or more of the following Multiple Sclerosis-Associated Protein Isoforms (MSPIs): MSPI-1, MSPI-2, MSPI-3, MSPI-4, MSPI-5.1, MSPI-6, MSPI-7, MSPI-8.1, MSPI-8.2, MSPI-9, MSPI-10, MSPI-11, MSPI-12.1, MSPI-12.2, MSPI-13, MSPI-14, MSPI-15, MSPI-16, MSPI-18, MSPI-20, MSPI-21, MSPI-22, MSPI-24, MSPI-25, MSPI-27, MSPI-28, MSPI-29, MSPI-30, MSPI-31, MSPI-32, MSPI-33.1, MSPI-33.2, MSPI-34, MSPI-36, MSPI-37, MSPI-39.1, MSPI-39.2, MSPI-40, MSPI-42.2, MSPI-43, MSPI-44, MSPI-47, MSPI-48.1, MSPI-48.2, MSPI-48.3, MSPI-49.1, MSPI-49.2, MSPI-51.1, MSPI-52.1, MSPI-52.2, MSPI-54, MSPI-55, MSPI-56, MSPI-58, MSPI-60, MSPI-62.2, MSPI-63, MSPI-64.1, MSPI-64.3, MSPI-65, MSPI-66, MSPI-67.1, MSPI-67.2, MSPI-69, MSPI-70, MSPI-72, MSPI-73, MSPI-75, MSPI-76, MSPI-77.2, MSPI-78.1, MSPI-78.2, MSPI-79, MSPI-80.1, MSPI-81, MSPI-82, MSPI-83, MSPI-84.1, MSPI-84.2, MSPI-86, MSPI-87, , MSPI-89.1, MSPI-90, MSPI-91.1, MSPI-92, MSPI-93.1, MSPI-93.2, MSPI-94, MSPI-95, MSPI-96, MSPI-97, MSPI-98, MSPI-99, MSPI-102.1, MSPI-102.2, MSPI-102.3, MSPI-104, MSPI-105.1, MSPI-105.2, MSPI-106, MSPI-107, MSPI-108, MSPI-110, MSPI-111, MSPI-112, MSPI-113, MSPI-114, MSPI-116, MSPI-117, MSPI-119, MSPI-120, MSPI-122, MSPI-123.1, MSPI-125.1, MSPI-125.2, MSPI-126.1, MSPI-126.2, MSPI-127.1, MSPI-128, MSPI-130, MSPI-131, MSPI-132, MSPI-133, MSPI-134, MSPI-135, MSPI-136, MSPI-137, MSPI-139, MSPI-140, MSPI-141.1, MSPI-141.2, MSPI-142, MSPI-143.1, MSPI-144, MSPI-145.2, MSPI-147.2, MSPI-148, MSPI-149, MSPI-150, MSPI-151, MSPI-152, MSPI-154.1, MSPI-154.2, MSPI-154.3, MSPI-155.1, MSPI-155.2, MSPI-155.3, MSPI-156.2, MSPI-158.2, MSPI-159, MSPI-160, MSPI-161, MSPI-163, MSPI-165.1, MSPI-165.2, MSPI-167, MSPI-168, MSPI-169.1, MSPI-169.2, MSPI-170, MSPI-171, MSPI-172, MSPI-173.2, MSPI-175, MSPI-176.1, MSPI-176.2, MSPI-177, MSPI-178, MSPI-179.1, MSPI-179.2, MSPI-180, MSPI-181.1, MSPI-181.2, MSPI-182, MSPI-183, MSPI-184, MSPI-185, MSPI-186, MSPI-187.1, MSPI-187.2, MSPI-188.2, MSPI-189, MSPI-191, MSPI-192.1, MSPI-192.2, MSPI-193, MSPI-194.1, MSPI-194.2, MSPI-195.1, MSPI-196, MSPI-197, MSPI-198, MSPI-199.1, MSPI-199.2, MSPI-200, MSPI-201, MSPI-202.1, MSPI-202.2, MSPI-203, MSPI-205, MSPI-206, MSPI-207, MSPI-208, MSPI-209, MSPI-210, MSPI-211, MSPI-212.2, MSPI-213, MSPI-215, MSPI-216, MSPI-217.3, MSPI-218, MSPI-219, MSPI-220, MSPI-221.1, MSPI-222.1, MSPI-222.2, MSPI-223.1, MSPI-223.2, MSPI-224.1, MSPI-225, MSPI-226, MSPI-228.1, MSPI-228.2,

MSPI-229.1, MSPI-229.2, MSPI-230, MSPI-231, MSPI-232, MSPI-233.1, MSPI-233.2, MSPI-234, MSPI-235, MSPI-236.1, MSPI-236.2, MSPI-237, MSPI-238, MSPI-239.1, MSPI-239.2, MSPI-240.1, MSPI-240.2, MSPI-241.1, MSPI-241.2, MSPI-242.1, MSPI-242.2, MSPI-243, MSPI-244, MSPI-245, MSPI-246.1, MSPI-246.2, MSPI-248, MSPI-250.2, MSPI-251, MSPI-254.1, MSPI-257.1, MSPI-257.2, MSPI-257.3, MSPI-260.1, MSPI-260.2, MSPI-261, MSPI-262.2, MSPI-265, MSPI-266, MSPI-267, MSPI-268.1, MSPI-270, MSPI-271, MSPI-273, MSPI-274, MSPI-276, MSPI-277, MSPI-279, MSPI-280.1, MSPI-280.2, MSPI-283.1, MSPI-283.2, MSPI-284.1, MSPI-284.2, MSPI-285, MSPI-289, MSPI-290, MSPI-291.1, MSPI-291.2, MSPI-292, MSPI-295, MSPI-298.1, MSPI-298.2, MSPI-299, MSPI-300, MSPI-301, MSPI-302, MSPI-303.2, MSPI-305, MSPI-306, MSPI-307, MSPI-308, MSPI-311.1, MSPI-311.2, MSPI-311.3, MSPI-313, MSPI-314, MSPI-316, MSPI-318, MSPI-319, MSPI-320, MSPI-322, MSPI-323.1, MSPI-323.2, MSPI-324, MSPI-325.1, MSPI-325.2

3. The method according to claim 1 or 2 where the body fluid is cerebrospinal fluid (CSF).

4. The method according to claim 2 or 3 where the abundance of the one or more MSPIs in the test sample is compared with the abundance of the one ore more MSPIs in a sample from one or more subjects free from Multiple Sclerosis, or with a previously determined reference range for that feature in subjects free from Multiple Sclerosis, or with the abundance at least one Expression Reference Feature (ERF) in the test sample.

5. The method according to any one of claims 2 to 4, wherein the step of quantitatively detecting comprises testing at least one aliquot of the first sample, said step of testing comprising:

(a) contacting the aliquot with an antibody that is immunospecific for a MSPI;

(b) quantitatively measuring the binding of the antibody and the MSPI; and

(c) comparing the results of step (b) with a predetermined reference range.

6. The method according to claim 5, wherein the step of quantitatively detecting comprises testing a plurality of aliquots with a plurality of antibodies cognate for a plurality of preselected MSPIs.

7. A pharmaceutical composition comprising a Multiple Sclerosis-Associated Protein Isoform (MSPI) as defined in claim 2, or a nucleic acid encoding an MSPI, and a pharmaceutically acceptable carrier.

8. The pharmaceutical composition according to claim 7, wherein the Multiple Sclerosis-Associated Protein Isoform (MSPI) is in recombinant form.

9. An antibody capable of immunospecific binding to a Multiple Sclerosis-Associated Protein Isoform (MSPI) as defined in claim 2.

10. The method according to claim 5 or 6 or an antibody according to claim 9, wherein the antibody is a monoclonal antibody.

11. The method according to claim 5 or 6 or an antibody according to claim 9 or 10, wherein the antibody is a chimeric antibody.

12. The method according to claim 5 or 6 or an antibody according to claim 9 or 10, wherein the antibody is a bispecific antibody.

13. The method according to claim 5 or 6 or an antibody according to claim 9 or 10, wherein the antibody is a humanised antibody.

14. The method according to claim 5 or 6 or an antibody according to any one of claims 9 to 13, wherein the antibody binds to the MSPI with greater affinity than to another isoform of the MSPI.

15. A kit comprising one or more antibodies as claimed in any one of claims 9 to 14 and/or one or more MSPIs as defined in claim 2, other reagents and instructions for use.

16. The kit of claim 15 for use in the screening or diagnosis of Multiple Sclerosis in a subject, for determining the stage or severity of Multiple Sclerosis in a subject, for identifying a subject at risk of developing Multiple Sclerosis, or for monitoring the effect of therapy administered to a subject having Multiple Sclerosis.

17. The kit according to claim 15 or 16 comprising a plurality of antibodies as claimed in any one of claims 9 to 14 and/or a plurality of MSPIs as defined in claim 2.

18. A pharmaceutical composition comprising a therapeutically effective amount of an antibody, or a fragment or derivative of an antibody according to any one of claims 9 to 14 and a pharmaceutically acceptable carrier.

19. A method of treating or preventing Multiple Sclerosis comprising administering to a subject in need of such treatment a therapeutically effective amount of an antibody as claimed in any one of claims 9 to 14.

20. A method of treating or preventing Multiple Sclerosis comprising administering to a subject in need of such treatment or prevention a therapeutically effective amount of one or more of the Multiple Sclerosis-Associated Protein Isoforms (MSPIs) as defined in claim 2 and/or a nucleic acid encoding said MSPIs.

21. A method of treating or preventing Multiple Sclerosis comprising administering to a subject in need of such treatment or prevention a therapeutically effective amount of a nucleic acid that inhibits the function of one or more of the Multiple Sclerosis-Associated Protein

Isoforms (MSPIs) as defined in claim 2.

22. The method according to claim 21, wherein the nucleic acid is an MSPI antisense nucleic acid or ribozyme.

23. A method of screening for agents that interact with one or more a Multiple Sclerosis-Associated Protein Isoforms (MSPIs) as defined in claim 2, fragments of MSPIs (MSPI fragment), polypeptides related to MSPIs (MSPI-related polypeptide), or MSPI-fusion proteins said method comprising:

(a) contacting an MSPI, a fragment of an MSPI, an MSPI-related polypeptide, or an MSPI-fusion protein with a candidate agent; and

(b) determining whether or not the candidate agent interacts with the MSPI, the MSPI fragment, the MSPI-related polypeptide, or the MSPI-fusion protein.

24. The method according to clain 23, wherein the determination of interaction between the candidate agent and the MSPI, MSPI fragment, MSPI-related polypeptide or MSPI-fusion protein comprises quantitatively detecting binding of the candidate agent and the MSPI, MSPI fragment, MSPI-related polypeptide or MSPI-fusion protein.

25. A method of screening for or identifying agents that modulate the expression or activity of one or more Multiple Sclerosis-Associated Protein Isoforms (MSPIs) as defined in claim 2, fragments of MSPIs (MSPI fragment), polypeptides related to MSPIs (MSPI-related polypeptide) or MSPI-fusion proteins comprising:

(a) contacting a first population of cells expressing the MSPI, MSPI fragment, MSPI-related polypeptide, or MSPI-fusion protein with a candidate agent;

(b) contacting a second population of cells expressing said MSPI, MSPI fragment, MSPI-related polypeptide, or MSPI-fusion protein with a control agent; and

(c) comparing the level of said MSPI, MSPI fragment, MSPI-related polypeptide, or MSPI-fusion protein or mRNA encoding said MSPI, MSPI fragment, MSPI-related polypeptide, or MSPI-fusion protein in the first and second populations of cells, or comparing the level of induction of a downstream effector in the first and second populations of cells.

26. A method of screening for or identifying agents that modulate the expression or activity of one or more Multiple Sclerosis-Associated Protein Isoforms (MSPIs) as defined in claim 2, fragments of MSPIs (MSPI fragment), polypeptides related to MSPIs (MSPI-related polypeptide) or MSPI-fusion proteins said method comprising:

(a) administering a candidate agent to a first mammal or group of mammals;

(b) administering a control agent to a second mammal or group of mammals; and

(c) comparing the level of expression of the MSPI, MSPI fragment, MSPI-related polypeptide or MSPI-fusion protein, or mRNA encoding said MSPI, MSPI fragment, MSPI-related polypeptide or MSPI-fusion protein in the first and second groups, or comparing the level of induction of a downstream effector in the first and second groups.

27. The method as claimed in claim 26, wherein the mammals are animal models for Multiple Sclerosis.

28. The method according to any one of claims 25 to 27, wherein administration of a candidate agent results in an increase in the level of said MSPI, MSPI fragment, MSPI-related polypeptide or MSPI-fusion protein, or mRNA encoding said MSPI, MSPI fragment, MSPI-related polypeptide, or MSPI-fusion protein, or said downstream effector in the first population of cells or mammals compared to the second population of cells or mammals.

29. The method according to any one of claims 25 to 27, wherein administration of a candidate agent results in a decrease in the level of said MSPI, MSPI fragment, MSPI-related polypeptide, or MSPI-fusion protein, or mRNA encoding said MSPI, MSPI fragment, MSPI-related polypeptide, or MSPI-fusion protein, or said downstream effector in the first population of cells or mammals compared to the second population of cells or mammals.

30. The method as claimed in claim 25 or 27, wherein the levels of said MSPI, MSPI fragment, MSPI-related polypeptide, or MSPI-fusion protein, or mRNA encoding said MSPI, MSPI fragment, MSPI-related polypeptide, or MSPI-fusion protein, or of said downstream effector in the first and second groups are further compared to the level of said MSPI, MSPI fragment, MSPI-related polypeptide or MSPI fusion protein, or mRNA encoding said MSPI, MSPI fragment, MSPI-related polypeptide or MSPI fusion protein in normal control mammals.

31. The method according to claim 30, wherein said mammals are human subjects having Multiple Sclerosis.

32. A method of screening for or identifying agents that modulate the activity of one or more of the Multiple Sclerosis-Associated Proteins Isoforms (MSPIs) as defined in claim 2, fragments of MSPIs (MSPI fragment), polypeptides related to MSPIs (MSPI-related polypeptide) or MSPI-fusion proteins said method comprising:

(a) in a first aliquot, contacting a candidate agent with the MSPI, MSPI fragment, MSPI-related polypeptide or MSPI fusion protein, and

(b) determining and comparing the activity of the MSPI, MSPI fragment, MSPI-related polypeptide or MSPI fusion protein in the first aliquot after addition of the candidate agent with the activity of the MSPI, MSPI fragment, MSPI-related polypeptide or MSPI fusion protein in a control aliquot, or with a previously determined reference range.

33. The method according to any one of claims 20 or 23 to 32, wherein the MSPI, MSPI fragment, MSPI-related polypeptide, or MSPI fusion protein is a recombinant protein.

34. The method according to any one of claims 23, 24 or 32, wherein the MSPI, MSPI fragment, MSPI-related polypeptide or MSPI fusion protein is immobilized on a solid phase.

35. A method for screening or diagnosis of Multiple Sclerosis in a subject or for monitoring the effect of an anti-Multiple Sclerosis drug or therapy administered to a subject, comprising:

(a) contacting at least one oligonucleotide probe comprising 10 or more consecutive nucleotides complementary to a nucleotide sequence encoding an MSPI as defined in claim 2 with RNA obtained from a biological sample from the subject or with cDNA copied from the RNA wherein said contacting occurs under conditions that permit hybridization of the probe to the nucleotide sequence if present;

(b) detecting hybridization, if any, between the probe and the nucleotide sequence; and

(c) comparing the hybridization, if any, detected in step (b) with the hybridization detected in a control sample, or with a previously determined reference range.

36. The method as claimed in claim 35, wherein step (a) includes the step of hybridizing the nucleotide sequence to a DNA array, wherein one or more members of the array are the probes complementary to a plurality of nucleotide sequences encoding distinct MSPIs.

37. A method of modulating the activity of one or more of the Multiple Sclerosis-Associated Protein Isoforms as defined in claim 2 comprising administering to a subject an agent identified by any one of claims 23 to 34.

38. A method of treating or preventing Multiple Sclerosis comprising administering to a subject in need of such treatment or prevention a therapeutically effetive dose of an agent that modulates the activity of one or more of the Multiple Sclerosis-Associated Protein Isoforms as defined in claim 2; whereby the symptoms of Multiple Sclerosis are ameliorated.

39. A method for identifying targets for therapeutic modulation of Multiple Sclerosis wherein the activity of one or more of the Multiple Sclerosis-Associated Protein Isoforms as defined in claim 2 is utilized as a measure to determine whether a candidate target is effective for modulation of Multiple Sclerosis.

FIGURE 1

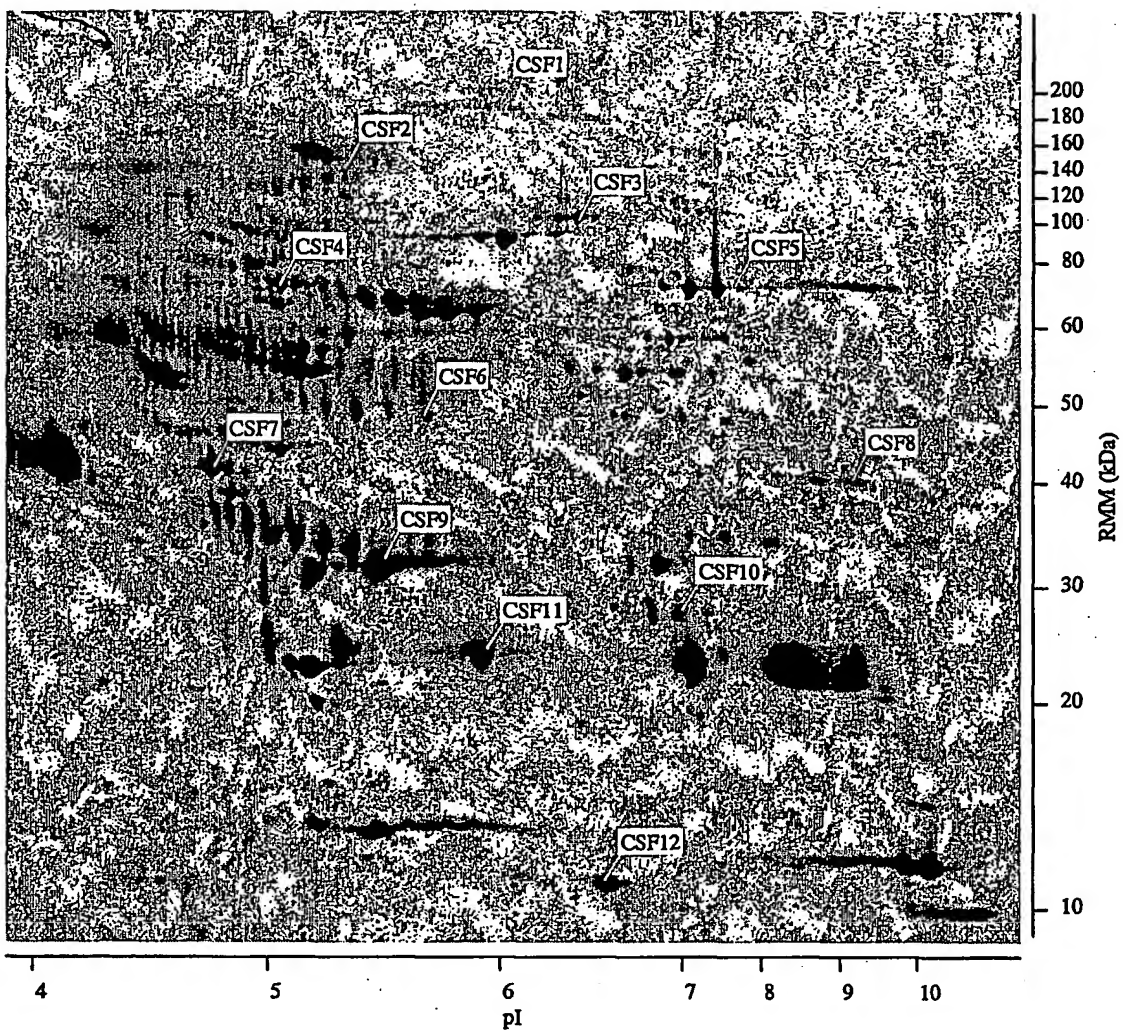
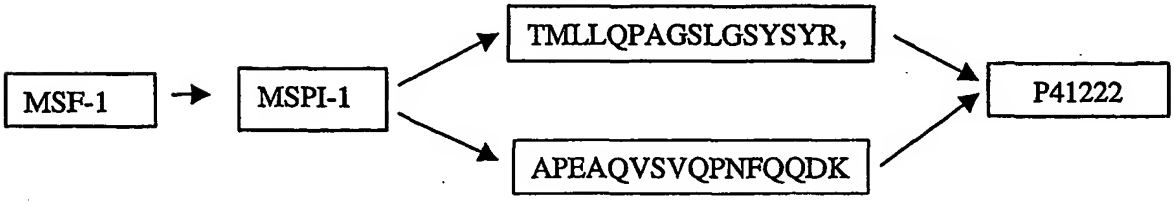


FIGURE 2



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 August 2002 (01.08.2002)

PCT

(10) International Publication Number
WO 02/059604 A2

(51) International Patent Classification[7]: G01N 33/564, A61K 39/00, A61P 25/00

(21) International Application Number: PCT/GB02/00330

(22) International Filing Date: 25 January 2002 (25.01.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/264,404	26 January 2001 (26.01.2001)	US
60/331,647	20 November 2001 (20.11.2001)	US

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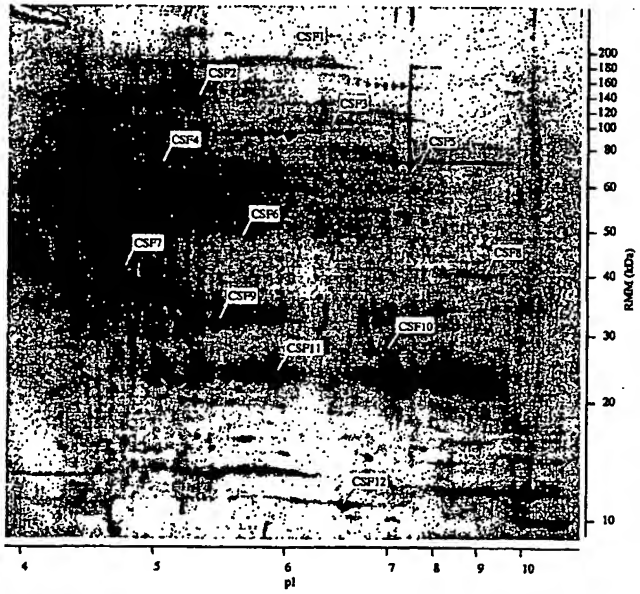
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

[Continued on next page]

(54) Title: PROTEINS, GENES AND THEIR USE FOR DIAGNOSIS AND TREATMENT OF MULTIPLE SCLEROSIS



(57) Abstract: The present invention provides methods and compositions for screening, diagnosis and prognosis of Multiple Sclerosis, for monitoring the effectiveness of Multiple Sclerosis treatment, identifying patients most likely to respond to a particular therapeutic treatment and for drug development. Multiple Sclerosis-Associated Features (MSFs), detectable by two-dimensional electrophoresis of body fluid e.g. cerebrospinal fluid are described. The invention further provides Multiple Sclerosis-Associated Protein Isoforms (MSPIs) detectable in body fluid e.g. cerebrospinal fluid, preparations comprising isolated MSPIs, antibodies immunospecific for MSPIs, and kits comprising the aforesaid.

WO 02/059604 A2

Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

(48) Date f publicati n f this corrected version:
21 November 2002

(15) Information about Correction:
see PCT Gazette No. 47/2002 of 21 November 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.